DRUG DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF SOME NOVEL HETEROCYCLIC COMPOUNDS AS ANTI-TUBERCULAR AGENTS

A Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY
CHENNAI - 600 032.

In partial fulfilment of the requirements for the award of the degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
Reg. No. 261215710
Under the Guidance of

DR. A.JERAD SURESH. M. PHARM., PHD., MBA.,

Principal, Prof & Head

COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003

APRIL 2014


DEDICATED TO
MY LOVEABLE
SISITER

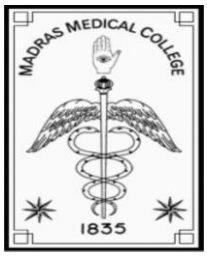
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003
TAMIL NADU

CERTIFICATE

This is to certify that the Dissertation entitled **"DRUG DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF SOME NOVEL HETEROCYCLIC COMPOUNDS AS ANTI-TUBERCULAR AGENTS"** submitted by the candidate with **Reg. No.261215710** in partial fulfilment of the requirements for the award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICAL CHEMISTRY** by The Tamil Nadu Dr. M.G.R. Medical University is a bonafide work done by him during the academic year 2013-2014.

Place: Chennai-03.

Date: (Dr. A.Jerad Suresh)



COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003
TAMIL NADU

CERTIFICATE

This is to certify that the Dissertation entitled **"DRUG DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF SOME NOVEL HETEROCYCLIC COMPOUNDS AS ANTI-TUBERCULAR AGENTS"** submitted by the candidate with **Reg. No.261215710** in partial fulfilment of the requirements for the award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICAL CHEMISTRY** under the guidance **Dr.A. Jerad Suresh M.Pharm., Ph.D., MBA**, Principal, Professor& head Department of Pharmaceutical chemistry. College of Pharmacy, Madras Medical College, Chennai.

Place: Chennai-03.

Date: (Dr. A.Jerad Suresh)

ACKNOWLEDGEMENT

"Gratitude makes sense of our past, brings peace for today and creates a vision for tomorrow"

I consider this as an opportunity to express my gratitude to all the dignitaries who have been involved directly or indirectly with the successful completion of this dissertation. The satisfaction that accompanies the successful completion of any task would be incomplete without mention of the people who made it possible with constant guidance, support and encouragement that crows all effort with success.

Many Thanks to **ALMIGHTY**, for it, He who began this work in me and carried it to completion. It is He who has blesses me with the people whose names I feel privileged to mention here.

It is with great pleasure that I place on record a deep sense of gratitude and heartfelt thanks to my guide **Prof. Dr. A. Jerad Suresh M.Pharm., Ph.D., MBA**, Principal, Head, Professor Department of Pharmaceutical chemistry, College of Pharmacy, Madras Medical College, Chennai, for their help, support and constant encouragement throughout the progress of this work. It was really a great experience working under them and their guidance, which was of immense help in my project work without which it would have been an unachievable task.

I am extremely happy to place on record my sincere gratitude and thanks to my esteemed teacher Dr**. V. Niraimathi, M.Pharm., Ph.D.**, Assistant Reader in Pharmacy, Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03 for her valuable suggestions, immense help and constant encouragement throughout the project work.

It’s a great pleasure for me to acknowledge my sincere thanks to all my teaching staff members **Mrs. P. G. Sunitha, Mrs. R. Priyadharshini, M.Pharm., Mr. M. Sathish, M. Pharm., Mrs. T. Saraswathy, M. Pharm., , M. Pharm.,** Tutors in Pharmacy, Department of Pharmaceutical Chemistry for their gracious support and encouragement in making this work more successful.

I extent my thanks to all non-teaching staff members Mr.R.sivakumar and Mr.baskar Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03.

I especially thankfull to all research scholar’s **Mr. K.M.Noorula**, **Mrs.R.devi** and **Ms.P.R.surya** Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03.

I express my heartiest thanks to **Dr. Kishore G Bhat,** Professor, Department of Microbiology, Maratha Mandal’s NGH Institute of Dental Sciences and Research Centre, Belgaum, Karnataka for his gracious support in carrying out the in-vitro evaluation of anti-tubercular activity.

The words are insufficient to thank my friends, David selvakumar.d, Sivasubramani.R.V and Pasupathi Raja.M who stood beside me in each and every step during my project and given me constant support.

I have no words to express my pleasure in thanking my dear friend’s murugan.v, kumar.k, magash kumar.M, mageshwari, sathiya suganya .S sathiya raj. senthil kumar.I,rama prahba,bakiyaraj.K and all others who are behind me supporting my endeavour.

I extend my cordial thanks to my seniors and to my juniors for their kind support and co-operation.

Most of all I would like to thank my beloved parents, brother, sister and my dearest friends for their priceless support, love and encouragement throughout the entire tenure of this course.

LIST OF ABBREVIATIONS

TLC	-	Thin Layer Chromatography
IR	-	Infrared
^{1}H-NMR	-	Proton Nuclear Magnetic Resonance
gm	-	Gram
hr	-	Hour
Sec	-	Seconds
R_f	-	Retention Factor
m.p	-	Melting Point
Mol.For	-	Molecular Formula
Mol.Wt	-	Molecular Weight
DMF	-	Dimethyl Formamide
DMSO	-	Dimethyl Sulphoxide
$^{\circ}$C	-	Degree Celsius
SEM	-	Standard Error Mean
m\e	-	Mass per charge ratio
STD	-	Standard
cfu mL^{-1}	-	Colony Forming unit per milliliter
UV	-	Ultraviolet
MIC	-	Minimum inhibitory concentration
mg\kg	-	Milligram per kilogram
µg	-	Microgram
b.w	-	Body weight
min	-	Minutes
TB	-	tuberculosis
Mdrtb	-	multi drug resistance tuberculosis

Introduction

PREFACE

Medicinal chemistry thus forms the chemical basis of therapeutics. Interdisciplinary science. It has been stated that "Medicinal chemistry concern the discovery, the development, the identification and interpretation of the mode of action of biologically active Compounds at the molecular level". Evidently it touches all branches of Chemistry and biology. Medicinal chemistry has interfaces with pharmaceutical chemistry, molecular pharmacology, bio-organic chemistry, and selective toxicity. Medicinal chemistry is a chemistry-based discipline, which involves aspects of biological, medical and pharmaceutical sciences.

It is concerned with the invention, discovery, design, identification and preparation of biologically active compounds, the study of their metabolism, the interpretation of their mode of action at the molecular level and construction of structural activity relationships by which they can be used as a medicine for the prevention mitigation and treatment of diseases.

The focus is mainly on organic medicinal substances. The organic drugs may be of natural or synthetic origin. The synthetic drugs have resulted by simple or mere modification of the structures of the natural drugs, or by pure synthesis. The other areas of collaboration in medicinal chemistry includes biology, computer aided drug design (CADD), 3-D QSAR, X-ray crystallography, metabolism, pharmacokinetics, legal and regulatory affairs, clinical franchise management, pharmaceutics and process research chemistry.

The practice of medicinal chemistry is devoted to the discovery, development of new agents for treating diseases. Most of the activity in this is devoted to new natural or synthetic organic compounds. The process of establishing a new drug is exceedingly complex and involves

the talents of people from a variety of disciplines, including chemistry, biochemistry, physiology, pharmacology, pharmaceutics and medicine. Medicinal chemistry is concerned mainly with the organic analytical and biologic interface of chemistry and biology. There is a considerable overlap with chemical aspects of this process. Thus it occupies a strategic position at the other disciplines.

INTRODUCTION

TUBERCULOSIS

Mycobacterium tuberculosis MTB, or TB (*tubercle bacillus*), in the past also called Phthisis or Phthisis pulmonalis, and colloquially, consumption or tisic, is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*.[1]Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It (identified by Koch in 1882) is the first bacteria recognized as causative agent for tuberculosis. [2] The dramatic importance of this still present illness is well known [3]. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit respiratory fluids through the air. [4] Over the past 200 years, tuberculosis was responsible for the death of 100 million people. [5]

The Mycobactenum tuberculosis complex constitutes a genetically closely related group, and its members, M. tuberculosis, Mycobactenum afncanum, Mycobactenurn bovis, and Mycobacterium microti, may be considered as subspecies of M. tuberculosis [6, 7]. The close relation between M. tuberculosis complex bacteria has been established by DNA-DNA hybridization (>95%) (l), multiple-locus enzyme electrophoresis [8], and Sequencing of 16s ribosomal RNA and housekeeping genes. [9] Furthermore, repetitive DNA elements, such as the insertion sequence IS6110 and the direct repeat have been found restricted to the M. tuberculosis complex, Nevertheless [10].the host range and pathogenicity of the M. tuberculosis complex species vary enormously. The natural reservoir of M. tuberculosis and M. afncanum is limited to humans. [11, 12] In contrast, the host range of M. bovis is very broad, and this species causes disease among a wide range of wild and domestic mammals as well as in humans. [13]

HISTORY OF TUBERCULOSIS

Tuberculosis has been present in humans since antiquity . [14] In order to assess the presence of tuberculosis in Pleistocene bison and the origin of tuberculosis in North America, 2 separate DNA extractions were performed by 2 separate laboratories on samples from the metacarpal of an extinct long-horned bison that was radiocarbon dated at 17,870 ± 230 years

before present and that had pathological changes suggestive of tuberculosis. [15]and researchers have found tubercular decay in the spines of Egyptian mummies dating from 3000–2400 BC. [16] *Phthisis* is a Greek word for *consumption*, an old term for pulmonary tuberculosis; [17] around 460 BC, Hippocrates identified phthisis as the most widespread disease of the times. [18]

Tuberculosis has claimed its victims throughout much of known human history. [19] It reached epidemic proportions in Europe and North America during the18th and 19th centuries, earning the sobriquet, ‘‘Captain Among these Men of Death.’’ [20] Then it began to decline. Understanding of the pathogenesis of tuberculosis began with the work of The´ophile Laennec at the beginning of the 19th century and was further advanced by the demonstration of the transmissibility of Mycobacterium tuberculosis infection by Jean-Antoine Villemin in 1865 and the identification of the tubercle bacillus as the etiologic agent by Robert Koch in 1882[21] . Clemens von Piquet developed the tuberculin skin test in 1907 and 3 years later used it to demonstrate latent tuberculosis infection in asymptomatic children. In the late 19th and early 20 centuries sanatoria developed for the treatment of patients with tuberculosis. [22] The rest provided there was supplemented with pulmonary collapse procedures designed to rest infected parts of lungs and to close cavities. [23] Public Health measures to combat the spread of tuberculosis emerged following the discovery of its bacterial cause. [24] BCG vaccination was widely employed following World War I. [25] the modern era of tuberculosis treatment and control was heralded by the discovery of streptomycin. [26, 27, 28]

TYPES:

Tuberculosis (TB) may be regarded in two categories: active disease or latent infection. The most common form of active TB is lung disease, but it may invade other organs, so called "extra pulmonary TB." [29]

Active TB Disease:

Active TB is an illness in which the TB bacteria are rapidly multiplying and invading different organs of the body [30]. The typical symptoms of active TB variably include cough, phlegm, chest pain, weakness, weight loss, fever, chills and sweating at night [31]. A person with active pulmonary TB disease may spread TB to others by airborne transmission of infectious

particles coughed into the air. [31][32][33] Depending on state or local public health regulations, the patent may be asked to take antibiotics under the supervision of a physician or other healthcare professional. [34] This program is called "Directly Observed Therapy" and is designed to prevent abandon mentorerratic treatment, which may result in "failure" with continued risk of transmission or acquired resistance of the bacteria to the medications, including the in famous multidrug resistant TB (MDR-TB). [35, 36]

Miliary TB:

Miliary TB is a rare form of active disease that occurs when TB bacteria find their way into the bloodstream. [37] In this form, the bacteria quickly spread all over the body in tiny nodules and affect multiple organs at once. This form of TB can be rapidly fatal. [38]

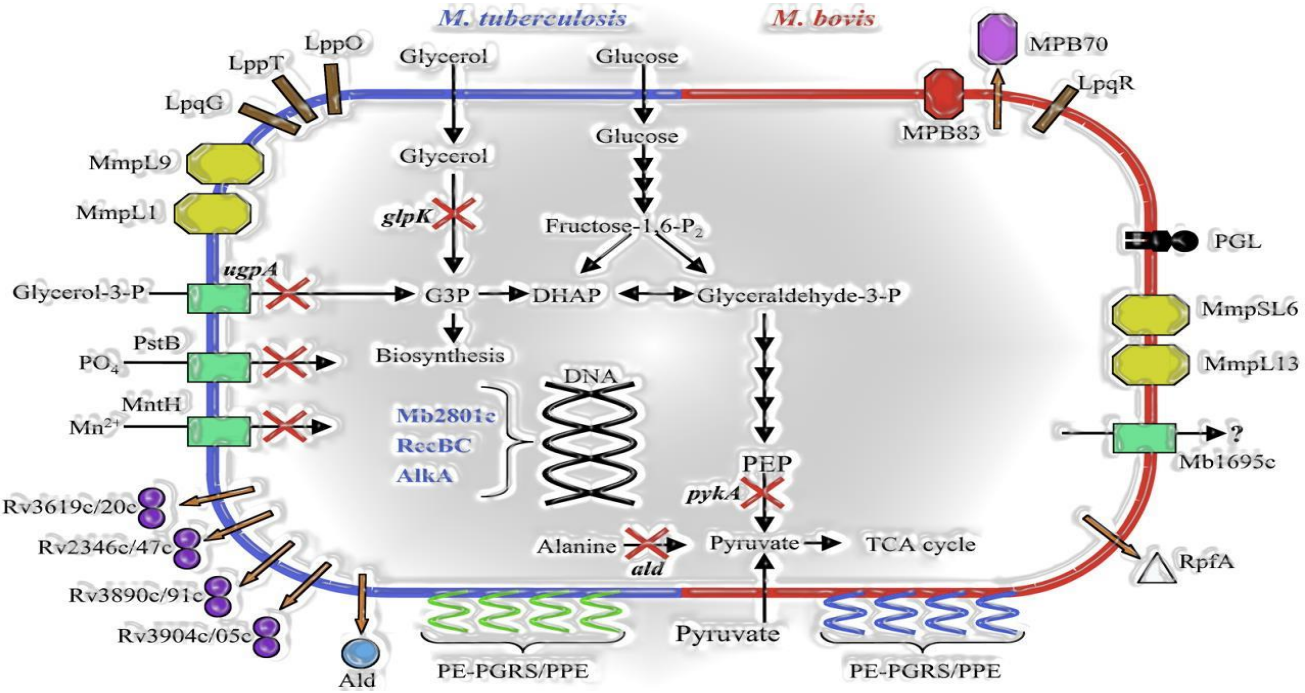
Latent TB Infection:

Many of those who are infected with TB do not develop overt disease. [39] They have no symptoms and their chest x-ray may be normal [40]. The only manifestation of this encounter may be reaction to the tuberculin skin test (TST) or interferon-gamma release assay (IGRA). However, there is an on-going risk that the latent infection may escalate to active disease. [41] increased Infection. [42]

MYCOBACTERIA

Mycobacteria are transition forms between bacteria and fungi. [43] The genus Mycobacterium belongs to the order Actinomycetales and the family mycobacteriaceae it is characterized by non-motile, non-sporulation rods that resist decolonization with acidified organic solvents and called as "acid fast". [43, 44]

Some mycobacterial species are pathogenic for humans among these, M. tuberculosis hominies and M. tuberculosis bovis.[45] Another variety M. africanum, endowed with characteristics intermediate between those of M. tuberculosis hominies and M. tuberculosis bovis is also pathogenic for humans. [46]



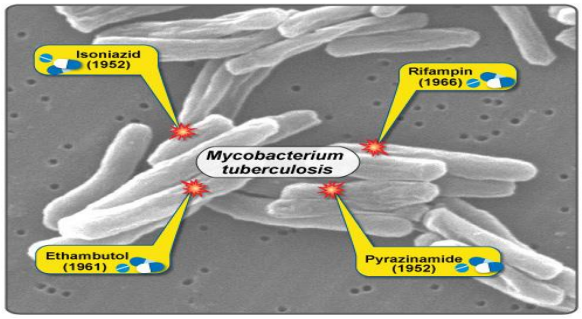
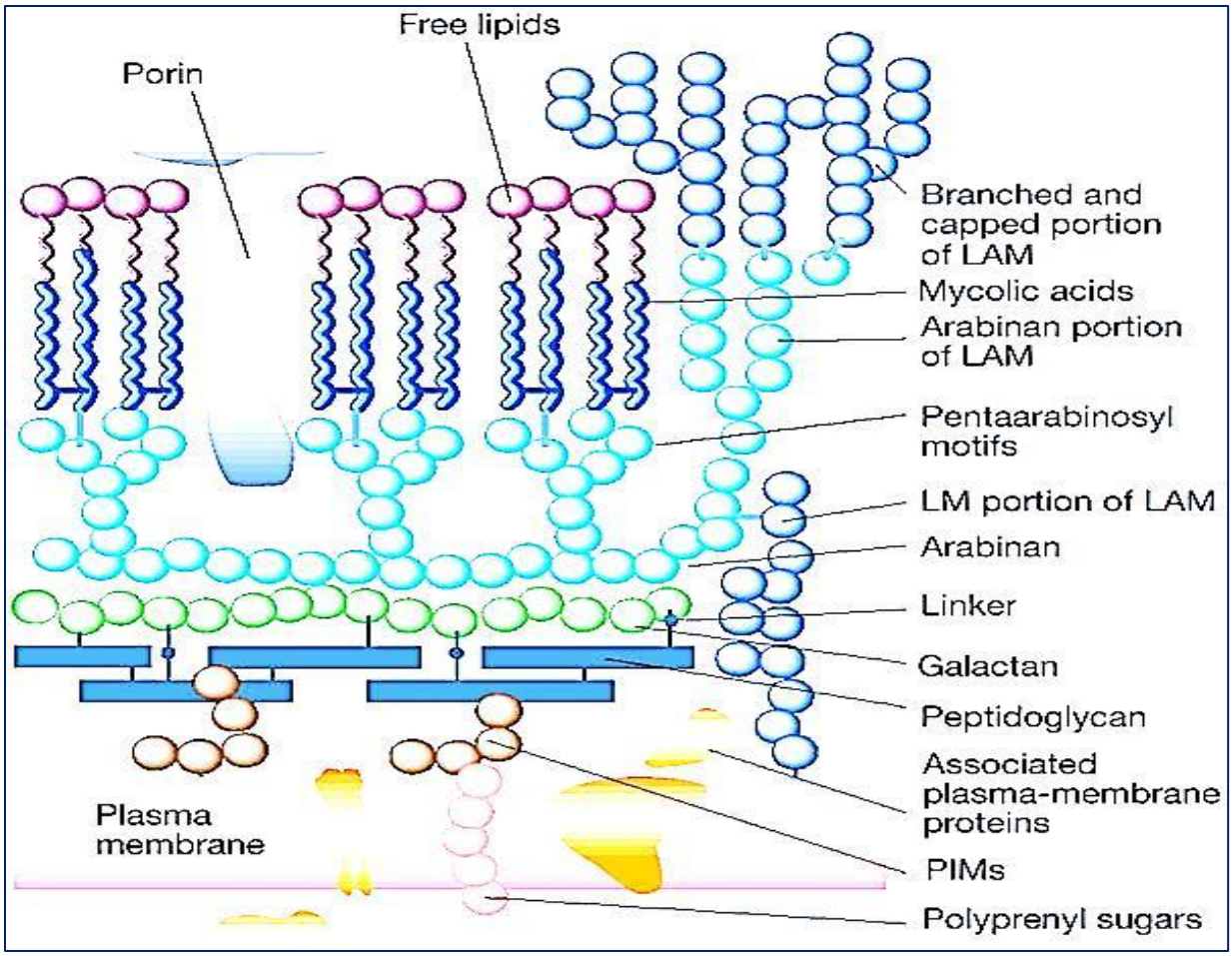


Figure1, 2[45] [46]

Mycobacterial cell wall:

The cell wall is a major virulence factor of *Mycobacterium tuberculosis* and contributes to its intrinsic drug resistance. [47] Recently, cryo-electron microscopy showed that the mycobacterial cell wall lipids form an unusual outer membrane. [48] Identification of the components of the uptake and secretion machinery across this membrane is critical for understanding the physiology and pathogenicity of *Tuberculosis* and for the development of better anti-tuberculosis drugs. [49,50] Although the genome of *Tuberculosis* appears to encode over 100 putative outer membrane proteins, only a few have been identified and characterized. [51] Here, we summarize the current knowledge on the structure of the mycobacterial outer membrane and its known proteins. Through comparison to transport processes in Gram-negative bacteria, we highlight several hypothetical outer membrane proteins of *Tuberculosis* awaiting discovery. [52, 53]

Figure-3[52, 53]



Mycobacteria have a complex cell envelope

Scientific interest in mycobacteria has been sparked by the medical importance of *Mycobacterium tuberculosis* and by properties that distinguish them from other microorganisms. [54] In particular, mycobacteria possess a remarkably complex cell envelope consisting of a cytoplasmic membrane and a cell wall, which constitutes an efficient permeability barrier and plays a crucial role in the intrinsic drug resistance and in survival under harsh conditions. [55] These microbes produce a fascinating diversity of lipids such as the mycolic acids, exceptionally long fatty acids that account for 30% to 40% of the cell envelope mass. [56] Mycolic acids are covalently linked to peptidoglycan via an arabino galactan polymer, a polysaccharide composed of arabinose and galactose subunits. [57] In a typical arrangement, the peptidoglycan network is substituted by linear galactan molecules, which bear several branched arabinose chains these branches end in four arabinose dimers, each forming the head group for two mycolic acid molecules. [58].

✓ **Mycobacterial outer membranes**

In 1982, Minnikin proposed that mycobacteria have a second lipid bilayer formed by an inner Leaflet of mycolic acids (covalently bound to the peptidoglycan) and an outer leaflet of free Lipids. [59] This proposal was the basis for a variety of models, suggesting an asymmetric outer Membrane-like lipid layer of exceptional thickness (≥10 nm). [60] Although freeze fracture Experiments supported the existence of this second membrane , electron microscopy of ultrathin sections failed to demonstrate the lipid bilayer structure, which was readily observed for the cytoplasmic membrane. [61] A breakthrough was achieved by the use of cryo-electron tomography (CET) and electron microscopy of ultrathin cryosections , techniques that abstain from harsh chemical treatment of biological samples CET revealed the native three-dimensional organization of the cell envelope of *Mycobacterium smegmatis* and *Mycobacterium bovis* BCG and disclosed the bilayer structure of the outer membrane. [62] While the lipopolysaccharide-containing outer membrane of Gram-negative bacteria consists of leaflet of different thicknesses electron microscopy showed that the mycobacterial outer membrane is approximately 8 nm thick and is morphologically symmetrical. [63] This finding, in combination with the observation that the mild detergent octyl β-glucoside permeabilizes the outer membrane of *M. smegmatis*, suggests that free

lipids with heterogeneous head groups are distributed over both leaflets in the mycobacterial outer membrane and are not restricted to the outer leaflet alone in contrast to all previous models [64].

✓ **The porin pathway across mycobacterial outer membranes**

While hydrophobic compounds can penetrate membranes by temporarily dissolving in the lipid bilayer, direct diffusion of water-soluble compounds across any lipid bilayer is too slow to support bacterial growth. [65] Thus, uptake of most hydrophilic solutes across the mycobacterial outer membrane likely requires some kind of transport proteins. [66] A strong argument in favour of this hypothesis is provided by the existence of porins such as Mycobacterium smegmatis (MspA) in mycobacteria. Porinsare defined as non-specific protein channels in bacterial outer membranes which enable the influx of hydrophilic solutes. [67] MspA was discovered as the major porin and the most abundant protein of *M. smegmatis* .Deletion of *MspA* reduced the outer membrane permeability towards glucose phosphate .metal ions and amino acids indicating that MspA represents the major general diffusion pathway in *M. smegmatis*. The loss of several Msp porins reduced the growth rate of *M. smegmatis* indicating that the influx of hydrophilic nutrients through porins are required for normal growth. [68] The requirement for fast nutrient uptake is likely not as stringent for *M. tuberculosis*, with a generation time of 24 hours compared to 3 hours for *M. smegmatis*. This might be the reason why *M. tuberculosis* does not have MspA homologues. [69] To assess the role of the porin pathway in *M. tuberculosis* and *M. bovis* BCG, the *mspA* gene of *M. smegmatis* was expressed in these species. [70] Even a very low number of MspA pores increased glucose uptake and accelerated growth of *M. bovis* BCG. [71] Further, both *M. bovis* BCG and *M. tuberculosis* became more susceptible to β-lactam antibiotics, isoniazid, ethambutol and streptomycin. [72] These results indicate that the very low efficiency and/or low number of endogenous pores contribute to the slow nutrient uptake and the intrinsic resistance of these organisms to drugs. [73]

Nevertheless, pore-forming proteins have been detected in *M. tuberculosis* and *M. bovis* BCG; further supporting that diffusion across the lipid bilayer of the outer membrane is too slow, at least for some solutes. [474] The C-terminal domain of one of these pore-forming proteins, OmpATb (Rv0899), has weak similarity to outer membrane proteins of the OmpA family of Gram-negative bacteria. Purified recombinant OmpATb was shown to form channels in lipid membranes and uptake of serine (but not of glycine) was reduced in the mutant of *M.tuberculosis*

lacking *ompATb.* [75] However, at pH 5.5, a 30-fold increase in transcription of *ompATb* was associated with decreased permeability of *M. tuberculosis* to serine. [76] Considering these conflicting results, it appears doubtful that the primary function of OmpATbin *M. tuberculosis* is that of a major porin .Another porin candidate, Rv1698, was identified as an outer membrane protein of *M.tuberculosis* with channel activity. [77] Heterologous expression of *rv1698* in an *M.smegmatis* porin mutant partially complemented the permeability defects of the strain. [78]

✓ **Structure of mycobacterial outer membrane proteins**

MspA is the only mycobacterial protein whose crystal structure has been solved. The structure has proven to be of immense value not only as a paradigm for a new class of proteins, but also for understanding the function of MspA , for elucidating its membrane topology, and for applications in nanotechnology . [79] The porin has an octameric goblet-like conformation with a single central channel 10 nm in length. This structure is different from that of trimetric porins in Gram-negative bacteria which have one pore per monomer and are approximately 5 nm long. The constriction zone of the octameric MspA channel consists of 16 aspartate residues (D90/D91) creating a high density of negative charges, which likely explain the cation preference of this porin. [80, 81] Due to its novel protein architecture, MspA became the founding member of a new class of outer membrane proteins which has more than 30 homologues in mycobacteria. More high-resolution structures are needed to identify unique characteristics of mycobacterial outer membrane proteins as well as features that are shared with proteins of Gram-negative bacteria. [82]

✓ **Energy-dependent uptake of nutrients across outer membranes**

Despite its important role in the uptake of some hydrophilic nutrients, the porin pathway is not efficient enough for (i) solutes of very low abundance (below 1 μM), such as iron, because small concentration gradients result in very low diffusion rates; and (ii) large solutes such as vitamin B12 exceed the size exclusion limit of most porin channels. Hence, uptake of these solutes across the outer membrane of Gram-negative bacteria requires active transport. [83] Substrates of energy-dependent transport systems bind with high affinity to surface receptors, Many with dissociation constants in the sub-Nano molar range. [84] In *E. coli,* energy is provided By the inner membrane complex ExbBD via the periplasmic protein TonB to multiple outer

Membrane receptors. [85]

Iron is highly limited to bacterial pathogens due to sequestration by the host and, therefore, needs to be acquired by active transport [86]. This is achieved by high affinity siderophores which are specifically taken up by outer membrane receptor proteins in Gram-negative bacteria .*E. coli* contains three major independent siderophore receptors in the outer membrane: FhuA,FepA and FecA. [87] Upon binding an iron-loaded siderophore, energy transferred by TonB initiates structural rearrangements in the transporter, releasing the siderophore into the periplasm where substrate binding protein shuttles it to a specific transporter of the ATP-binding cassette transporter family in the inner membrane. [88]

M. tuberculosis produces two salicylate-derivedsiderophores. The more polar carboxymycobactin is released into the medium, whereas the less polar mycobactin remains associated with the cell. [89] In this bacterium, an ABCtransporter composed of the proteins IrtA and IrtB is required for export of siderophores across the inner membrane . [90] Uptake of iron-loaded carboxymycobactin is not energy-dependent, leading to the hypothesis that siderophores might diffuse through porins. [91]

- ✓ **Uptake of hydrophobic compounds across outer membranes**

Nikaido and co-workers have shown that diffusion rates through the water-filled channels ofporins drop drastically with increasing solute hydrophobicity . [92] Both direct diffusion of anionic fatty acids through lipid membranes and alternative ‘flip-flop’ movement of protonated fatty acids through membrane are slow. [93] These findings explain why bacteria and eukaryotes have evolved proteins for fatty acid uptake across membranes. For example, the outer membrane protein FadL mediates energy-independent uptake of fatty acids by *E. coli.* [95]

Considerable circumstantial evidence suggests that *M. tuberculosis* uses lipids as a carbon source after the onset of the adaptive immune response in mice. However, no FadL homologue is apparent in mycobacteria, and the mechanism by which fatty acids cross the mycobacterial outer membrane is unknown. [96] Identification of an outer membrane fatty-acid transporter will be important for understanding the physiology of *M. tuberculosis* in the human host and might shed light on the types of lipids used by this microbe. In addition to fatty acids, cholesterol is another hydrophobic compound which appears to be used by *M. tuberculosis* asa carbon source. [97, 98] Interestingly, the *mce4* operon is required for efficient uptake of cholesterol and encodes some proteins (Mce4A, Mce4B, Mce4C, Mce4D and Mce4F) that have been proposed to be outer

membrane proteins, based on secondary structure predictions and other characteristics. [99] They might form an outer membrane channel to enable cholesterol to enter the cell.

- ✓ **Efflux processes**

M. tuberculosis is intrinsically resistant to many antibiotics due to the formidable permeability barrier established by the outer membrane, in synergy with other resistance mechanisms such as multi-drug efflux. [100] considering that its genome encodes 69 putative drug efflux pumps, it is not surprising that all current tuberculosis drugs are substrates of efflux. In Gram negative bacteria, only efflux across both membranes is an effective resistance mechanism, and we expect a similar situation to occur in mycobacteria [101]. The major drug efflux system of *E. coli* is a tripartite pump consisting of an inner-membrane transporter protein (AcrB), aperiplasmic adapter protein (AcrA) and an outer membrane channel (TolC). [102] E. *coli tolC*mutants are highly susceptible to a wide variety of toxic compounds .Although TolC homologues are ubiquitous among Gram-negative bacteria; they do not seem to exist in mycobacteria. [103] hence, we hypothesize that *M. tuberculosis* might have an outer membrane channel protein that connects to inner membrane pumps, allowing for efficient efflux across the two membranes. [104] this hypothesis is supported by the observation that overexpression of *rv0194*, a gene encoding an inner-membrane ABC transporter of *Tuberculosis*, increased resistance of *M. bovis* BCG to ampicillin. [105] because targets of β-lactam antibiotics are located in the periplasm, increased resistance must result from efflux across the outer membrane. [106] a connection of an outer membrane channel with an inner membrane efflux pump similar to the drug efflux systems in Gram-negative bacteria would also provide the energy required for efflux against the concentration gradient. [107,108] Discovery of a TolC-like protein would represent a major breakthrough in our understanding of drug efflux in *M. tuberculosis* [109].

- ✓ **Other putative outer membrane protein**

In the previous sections, we have highlighted a few transport processes requiring outer membrane proteins. [110] yet, many other functions as described in Gram-negative bacteria are completed by proteins embedded in the outer membrane. [111] we propose that functionally equivalent proteins exist in mycobacteria. For example, yet is required by *E. coli* to correctly insert proteins into the outer membrane. [112] Conditional depletion of the homologous Omp85 in *Neisseria gonorrhoeae* results in periplasmic accumulation of miss folded proteins. [113] Mycobacteria might possess a functional homologue of protein insertion machinery in the

outer membrane. Similarly, Gram-negative bacteria require an outer membrane protein, Imp, to insert lipopolysaccharide (LPS) into the outer leaflet of the outer membrane. [114] such membrane assembly proteins are likely also required for the many different lipids in the outer leaflet of mycobacterial outer membranes. [115] For example, transport of the phthiocerol di mycocerosates (PDIMs) requires the inner membrane transporter MmpL7 and the lipoprotein LppX, but it is unknown how PDIMs cross the outer membrane to reach the cell surface. [116] Mycobacteria produce capsules and biofilms, but it is unknown how the materials for these extracellular structures are secreted to the cell surface. [117] In *E. coli*, capsular material is Trans locating across the outer membrane by the Wza protein. [118] given the requirement of extracellular structures for the survival of *M. tuberculosis* during infection we propose that secretion machinery for biofilm and capsular materials in the outer Membrane also exists. [119]

PATHOPHSIOLOGY:

When many infectious units of 1-3 bacilli are inhaled, a phenotypically hardy bacillus is likely to be among them. In addition, the alveolar macrophages apparently vary in their capacity to destroy bacilli. [120]

Staining for acid-fast bacilli is very useful for demonstrating *M. tuberculosis*

- Histologically, tuberculosis displays exudative inflammation
- proliferative inflammation
- and productive inflammation[121,122]

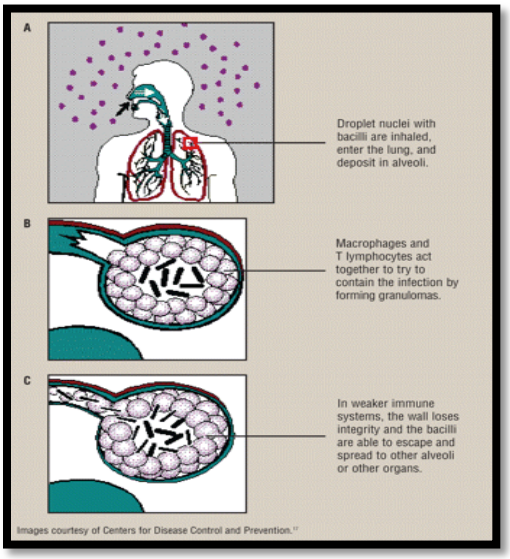


Figure-4[123]

Once inhaled, the infectious droplets settle throughout the airways. The majority of the bacilli are trapped in the upper parts of the airways where the mucus-secreting goblet cells exist. [123] the mucus produced catches foreign substances, and the cilia on the surface of the cells constantly beat the mucus and its entrapped particles upward for removal. This system provides the body with an initial physical defense that prevents infection in most persons exposed to tuberculosis. [124]

TRANSMISSION:

M. tuberculosis is carried in airborne particles, called droplet nuclei, of 1–5 microns in diameter. Infectious droplet nuclei are generated when persons who have pulmonary or laryngeal TB disease cough, sneeze, shout, or sing. [125] Depending on the environment, these tiny particles can remain suspended in the air for several hours. *M. tuberculosis* is transmitted through the air, not by surface contact. Transmission occurs when a person inhales droplet nuclei containing *M. tuberculosis*, and the droplet nuclei traverse the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lungs. [126]

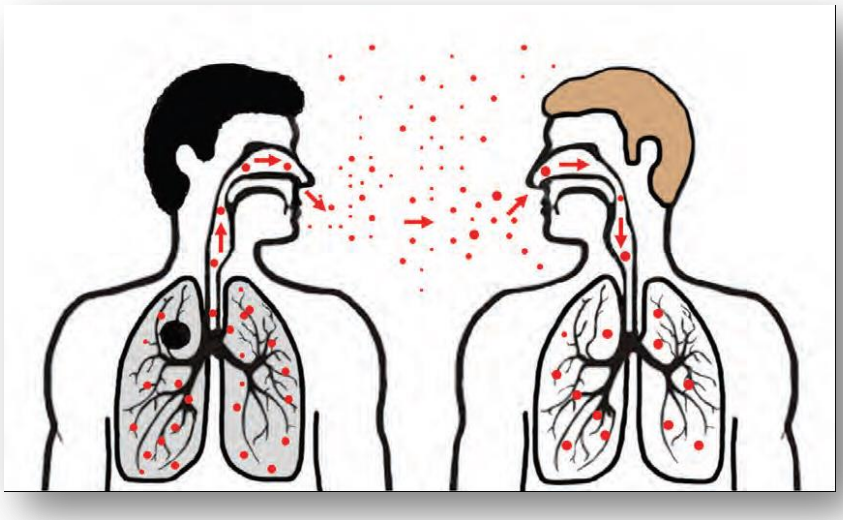


Figure-5[126]

TB is spread from person to person through the air. The dots in the air Represent droplet nuclei containing tubercle bacilli.

Factors that Determine the Probability of Transmission of *M. tuberculosis:* [127]
Table-1

FACTOR	DESCRIPTION
Susceptibility	Susceptibility (immune status) of the exposed individual
Infectiousness	Infectiousness of the person with TB disease is directly related to the Number of tubercle bacilli that he or she expels into the air. Persons who expel many tubercle bacilli are more infectious than patients who expel few or no bacilli
Environment	Environmental factors that affect the concentration of *M. tuberculosis* Organisms
Exposure	Proximity, frequency, and duration of exposure

PATHOGENESIS AND EPIDEMIOLOGY

Infection occurs when a person inhales droplet nuclei containing tubercle bacilli that reach the Alveoli of the lungs. These tubercle bacilli are ingested by alveolar macrophages; the majority of these bacilli are destroyed or inhibited. [127]

Tuberculosis is sometimes an acute but more frequently a chronic communicable disease that derives its character from several properties of the tubercle bacillus, which in contrast with many common bacterial pathogens, multiplies slowly, does not produce exotoxins, and does not stimulate an early reaction from the host. The tubercle bacillus is also an intracellular parasite, living and multiplying inside macrophages. [127]

Chronic pulmonary tuberculosis in adults may be due to reactivation of the primary infection or to exogenous reinfection. A typical characteristic of tuberculosis is the formation in the infected tissue nodular formations called tubercles, which can have different sizes and different modes of diffusion, giving rise to various clinical forms called military, infiltrate, lobar tuberculosis, and so on. The disease progresses by means of ulceration, caseation and cavitation,

with bronchogenic spread of infectious material. Healing may occur at any stage of the disease by processes of resolution, fibrosis, and calcification. [128]

Control of the disease has been achieved in part through mass vaccination with BCG (the bacillus of Calmette and Guerin, an attenuated strain of M. tuberculosis bovis).

DRUG-RESISTANT TB (MDR AND XDR):

Drug-resistant TB is caused by *M. tuberculosis* organisms that are resistant to the drugs normally used to treat the disease (Figure). Drug-resistant TB is transmitted in the same way as drug-susceptible, and is no more infectious than drug-susceptible TB. [129] however, delay in the recognition of drug resistance or prolonged periods of infectiousness may facilitate increased transmission and further development of drug resistance. [130]

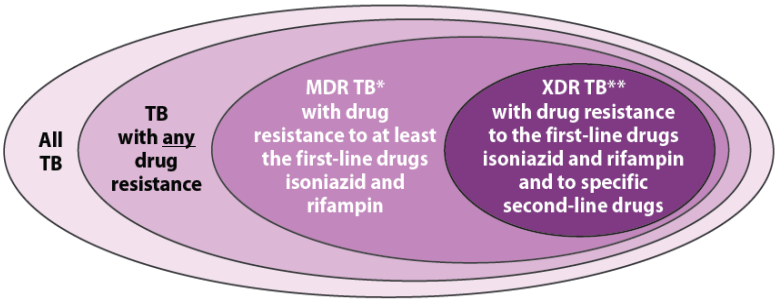


Figure-6[130]

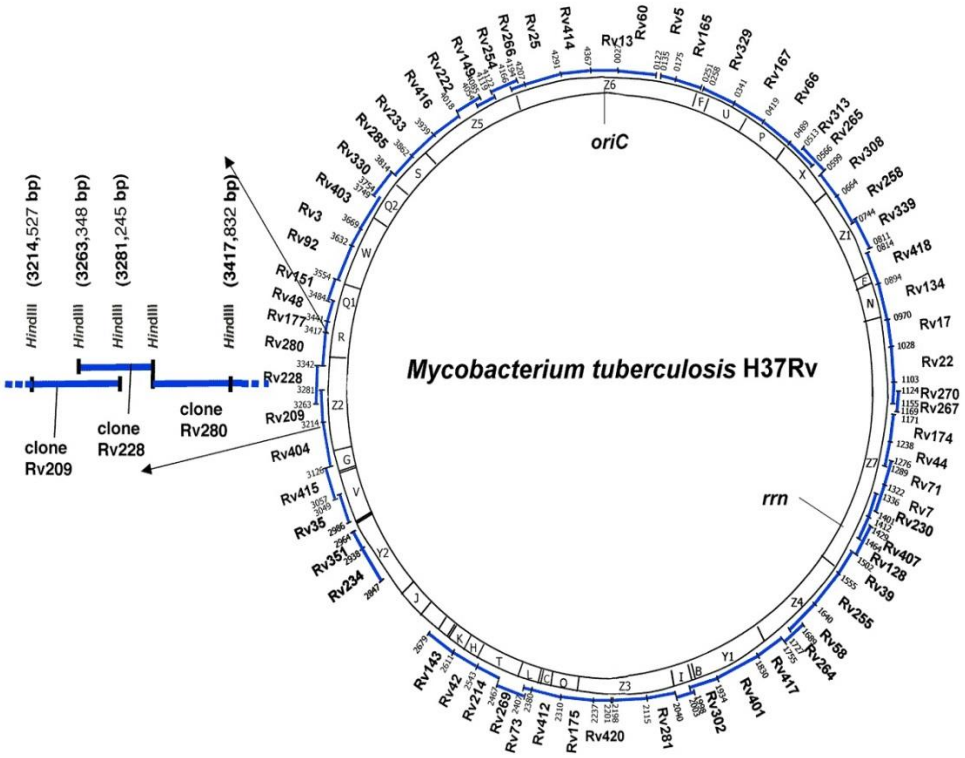
Drug-Resistant Tuberculosis

GENOME:

Countless millions of people have died from tuberculosis, a chronic infectious disease caused by the tubercle bacillus. The complete genome sequence of the best-characterized strain of *Mycobacterium tuberculosis*, H37Rv, has been determined and analysed in order to improve our understanding of the biology of this slow-growing pathogen and to help the conception of new prophylactic and therapeutic interventions. [131,132] The genome comprises 4,411,529 base pairs, contains around 4,000 genes, and has a very high guanine + cytosine content that is reflected in the biased amino-acid content of the proteins. *M. tuberculosis* differs radically from other bacteria in that a very large portion of its coding capacity is devoted to the production of enzymes involved

in lipo genesis and lipolysis, and to two new families of glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation. [133]

Figure-7[133]



H37Rv.....GSGAPGGAGGAAGLWGTGGAGGAGGSSAGGGGAGGAGGAGGWLLGDGGAGGIGGAST...
BCG.....GSGAPGGAGGAAGLWGTGGA----------GGAGGWLLGDGGAGGIGGAST...

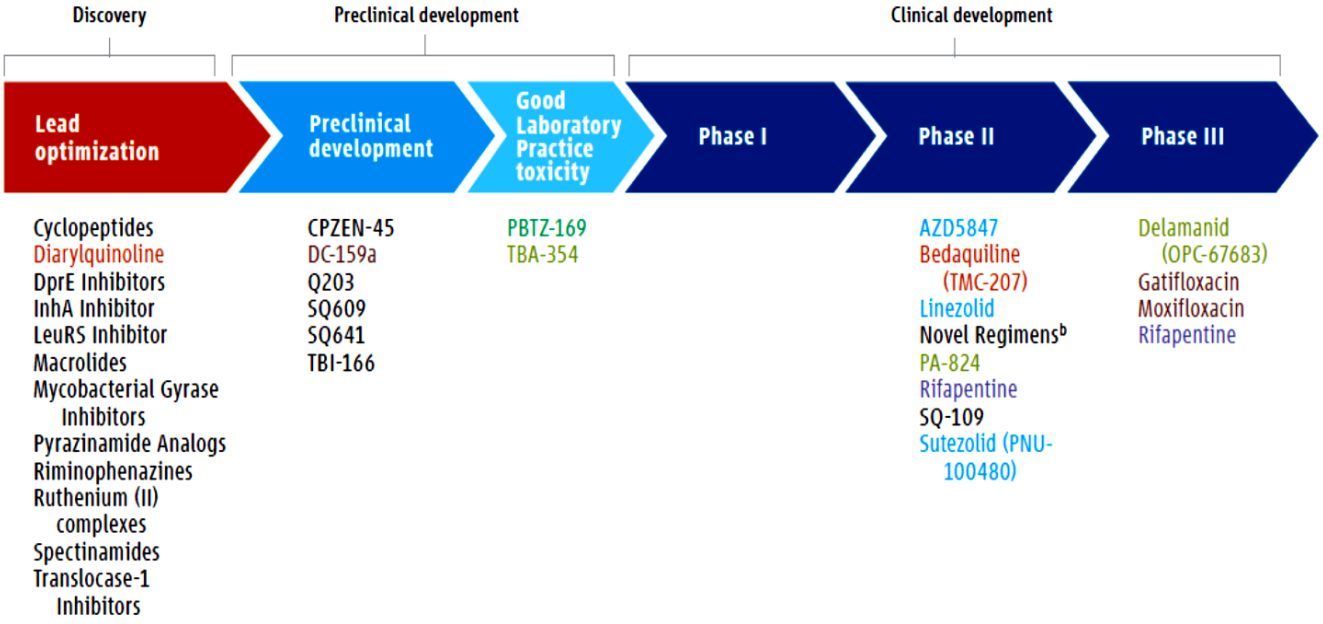
This codon's of H37RV genome sequence.

CURRENT STATUS OF TUBERCULOSIS:

Tuberculosis (TB) continues to remain one of the most pressing health problems in India. India is the highest TB burden country in the world, accounting for one fifth of the global incidence - an estimated 1.96million cases annually. Approximately 2.9million people die from tuberculosis each year worldwide; about one fifth of them in India alone .Nearly 500,000 die from the disease – more than1000 per day–one every minute. The disease is a major barrier to social and economic development. [134]

An estimated 100 million workdays are lost due to illness. The society and the country also incur a huge cost due to TB–nearly US$ 3 billion in indirect cost and US$ 300 million in direct costs. The situation is more complicated considering that disproportionately affects the young population in India. TB mortality in the country has reduced from an estimated 42 per lakh population in 1990 to 28 per lakh population in 2006, and the prevalence of TB in the country has reduced from 568 per lakh population in 1990 to 283 per lakh population by the year 2012. [135]

NEW PIPELINE FOR TB DRUG'S SYSTHESIS: [136]

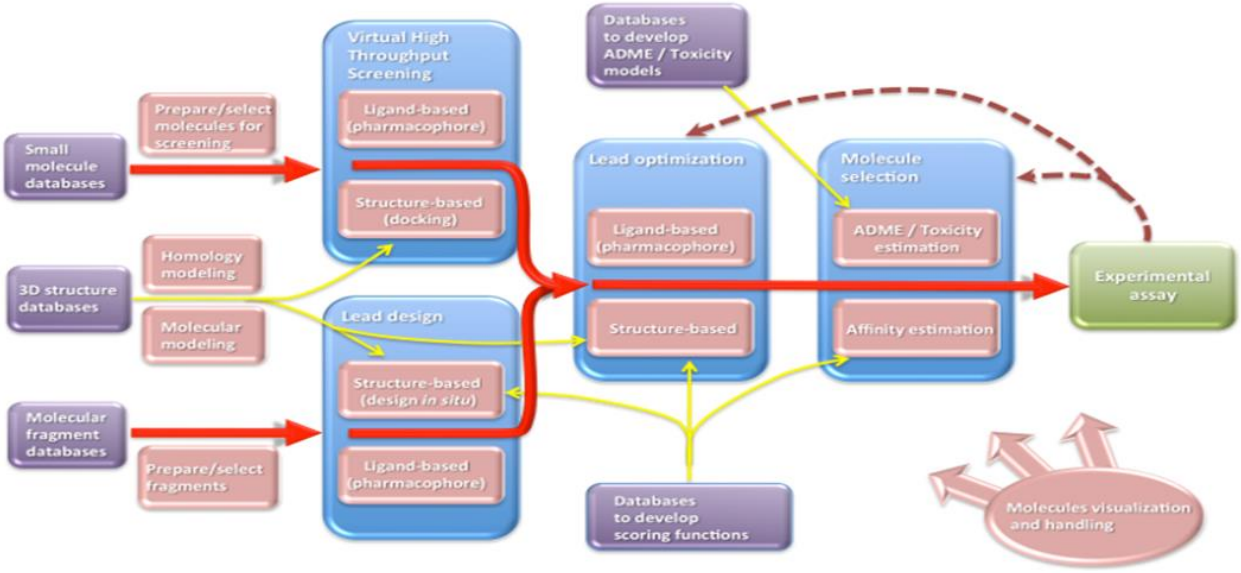


THE DRUG DISCOVERY PROCESS:

Drug discovery process basically is a patient oriented science, where researchers strive to improve the existing drugs or invent a totally new chemical entity, which should be ideally more potent than any existing drug of a similar category. If not, then at least it should be safer than those existing. This process is a very time consuming and expensive activity, calling for the expertise of many eminent researchers. It takes nearly 12-14 years of exhaustive research and a huge amount of

financial investment for the discovery of a single drug. Right from the chemical synthesis to its clinical development and finally formulating it to a suitable form. Failure at any stage would mean a huge loss for the company. Hence, a lot of planning is required even before the project is underway. Recently, with the use of technology the process is becoming a less risky business, because of the ability of the computers to predict the possible outcomes. This will surely reduce the efforts in fruitless directions. [138]

Figure-8[138]



***TARGET IDENTIFICATION AND VALIDATION*:**

Target identification involves choosing the right target which binds with the small molecule. A target is generally a single molecule, such as gene or protein, which involved in the pathogenesis of a particular disease. The target should be selected in such a way that it could potentially interact with and be affected by the drug molecule. [139]

LEAD IDENTIFICATION:

Lead identification is the search for the molecule or lead compound that may act on the target to alter the disease course. The ways to find a lead compound are as follows:

De novo:

De novo design refers to a computer assisted molecular design that supports drug discovery by suggesting novel chemo types and compound modifications for lead structure optimization. [140]

High-throughput Screening:

This process is the most common way that leads are usually found. Advances in robotics and computational power allow researchers to test hundreds of thousands of compounds against the target to identify any that might be promising. Based on the results, several lead compounds are usually selected for further study. [141]

Biotechnology:

This field involves genetically engineering living systems to produce disease-fighting biological molecules. [142]

LEAD OPTIMIZATION:

Lead compounds that survive the initial screening are then "optimized," or altered to make them more effective and safer. By changing the structure of a compound, its properties could be altered. For example, they can make it less likely to interact with other chemical pathways in the body, thus reducing the potential for side effects. [143] Hundreds of different variations or "analogues" of the initial leads are made and tested. Teams of biologists and chemists work together closely: The biologists test the effects of analogues on biological systems while the chemists take this information to make additional alterations that are then retested by the biologists. The resulting compound is the candidate drug. [144]

New techniques such as magnetic resonance imaging, X-ray crystallography, along with powerful computer modeling techniques helps us to visualize the target in three dimensions and design potential drugs to more powerfully bind to the parts of the target where they can be most effective. [145]

DRUG DESIGN:

For the pharmaceutical industry, the number of years to bring a drug from discovery to market is approximately 12-14 years and costing up to $1.2 - $1.4 billion dollars. Traditionally, drugs were discovered by synthesizing compounds in a time-consuming multi-step processes against a battery of ***in vivo*** biological screens and further investigating the promising candidates

for their pharmacokinetic properties, metabolism and potential toxicity. [146] Such a development process has resulted in high attrition rates with failures attributed to poor pharmacokinetics (39%), lack of efficacy (30%), animal toxicity (11%), adverse effects in humans (10%) and various commercial and miscellaneous factors. Today, the process of drug discovery has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, ***de novo*** design, ***in vitro***, ***in silico*** ADMET screening and structure-based drug design. [147]

There are two major types of drug design.

1. LIGAND BASED DRUG DESIGN
2. STRUCTURE BASED DRUG DESIGN

IN-SILICO DRUG DESIGN:

In-silico methods can help in identifying drug targets via bioinformatics tools. [148] they can also be used to analyses the target structures for possible binding/active sites, generate candidate molecules, check for drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics.

The use of computers and computational methods permeates all aspects of drug discovery today and forms the core of structure-based drug design. High Performance computing, data management software and internet are facilitating the access of huge amount of data generated and transforming the massive complex biological data into a workable knowledge in modern day drug discovery process. [149] The use of complementary, experimental and informatics techniques increases the chance of success in many stages of drug discovery process, from the identification of novel targets and elucidation of their functions to the discovery and development of lead

compounds with desired properties. Computational tools offer the advantage of delivering new drug candidates more quickly and at lower cost. Major roles of computation in drug discovery are;

1. Virtual screening and de novo design
2. *Insilco* ADME/Toxicity production
3. Advanced methods for determining protein ligand binding [160]

LIGAND-BASED RUG DESIGN:

Ligand based approaches commonly consider two or three dimensional chemistry, shape, electrostatic and interaction points (e.g. Pharmacophore modelling) to assess similarity.

STRUCTURE BASED DRUG DESIGN (SBDD) :

Structure based design attempts to use the three dimensional (3D) protein structure to predict which ligands will bind to the target. Structure-based approaches, of which the best known is docking, require a protein structure or homology model as a starting point. SBDD is an iterative process, in which macromolecular crystallography has been the predominant technique used to elucidate the 3D of drug targets. Although both nucleic acids and proteins are potential drug targets, by far the majority of such targets are proteins. Given that many proteins undergo considerable conformational change upon ligand binding, it is important to design drugs based on the crystallographic structures of protein-ligand complexes. [161]

Crystallography has been successfully used in the de novo design of drugs, but its most important use has been, and will continue to be, in lead optimization .It is important to note that what is being optimized is the affinity and specificity of compounds to their drug target.

Lead optimization is a multi-step process that can be summarized as follow:

- Expression and purification of the protein of interest. Crystallisation of the protein in the presence of a ligand, which can be a non-hydrolysable substrate or can come from a biochemical or a cell-based screen.
- Ligands can be low affinity compound fragments or scaffold. They are generally a collection of basic chemical building blocks, each with a molecular weight of less than 200 Daltons. If the screen identifies several promising ligands, each with a unique scaffold, determine the structures of the drug target with as many of these as possible.
- One or more ligands have been determined and refined, analysis of each structure will reveal sites on the ligand that can be optimized to enhance potency to the drug target. This can be accomplished by redesigning the ligand with greater hydrophobic, hydrogen-bonding and electrostatic complementary to the molecular target. A high affinity lead makes the drug design process simple and intuitive.
- After the ligands have been designed they should be chemically synthesized. It is prudent to synthesis five to ten compounds around the proposed ligand to obtain structure-activity relationship (SAR) data.
- Once the synthesized compounds are purified, they are tested in a relevant biochemical or cell-based assay to determine whether or not the design was successful. [162,163]

CYCLOPROPANE SYNTHASE CmaA2

The crystal structures of cyclopropane synthase CmaA2 is closely related with root mean square deviation (RMSD) between the Ca atoms of the core region of less than 0.7Å The core region which contains a seven stranded b-sheet which are all parallel apart from b7 which runs antiparallel. The α-helices flank each side of the sheet and run in the same N- to C- orientation. The two long α-helices lie adjacent to the C- terminal ends of the b-sheet, which encloses SAM/SAH cofactor binding site. In cyclopropane synthase the overall polypeptide fold are similar to other SAM-Mtases in the protein database, such as catechol-O-methyltransferases and DNA methyltransferases. [164]

Despite the low sequence similarity level to other methyltransferases, the cofactor binding sites are conserved in sequence and structure.

The structure of cyclopropane enzymes is revealed by a tunnel approximately 15Å by 10 Å wide which extends from the surface of the protein to the cofactor binding site. The tunnel is exclusively lined with hydrophobic residues and believed to be the binding site for the acyl substrate and is virtually identical in the three enzymes CmaA1, CmaA2 and PcaA. Structure of CmaA2 in complex with SAH and the lipid like detergents cetyltrimethylammonium bromide (CTAB) or didecyldimethylammonium bromide (DDDMAB) have applied this concept. The important interactions between the acyl chain and the protein includes Leu192, Ile169, Phe200, Ile195, Leu205, Leu236, Tyr232, Leu278 and Phe273 it reveals that the reactive group may sit in the active binding site and the length of the acyl chain which these enzymes may accept apart from the interactions of the co-crystal structure with the lipid. [165]

It is clear that the active binding sites for the three cyclopropane synthase structures are similar and the residues that bind the SAM cofactor as well as those which are important in catalysis are conserved shows that these enzymes operate through the same reaction mechanism.[18] While comparing the structures of CmaA2 and PcaA the two enzymes which act at the proximal position to produce trans and cis cyclopropane rings respectively and reveals only a minor differences between the two structures. [167]

The cyclopropane synthase CmaA2 and other enzyme are the part of FASII pathway for the biosynthesis of mycolic acids in mycobacteria and these enzymes acts on a long acyl chain

which linked to acyl carrier protein (AcpM). The mechanism for distal versus proximal substrate specificity is based on the differing modes of binding of acyl-AcpM.

Recent studies shows the cyclopropane synthases of M.tuberculosis is considered as a novel class of persistence genes and the need of new inhibitors for the persistent phase of tuberculosis infection and the absence of the cyclopropanated lipids in human results cyclopropane synthases as an attractive target for the new drug development. Despite the apparent non-redundancy of the CmaA2 and other cyclopropane synthases, the similarity of this family of enzymes in the mode of binding substrates and in their catalytic mechanism is very clear. This makes the prospect of a single drug effectiveness against multiple targets is highly possible, so that the chance for the development of drug resistance is less. [168]

MODIFICATIONS INVOLVING METHYL TRANSFER FROM SAM

Experiments in which mycobacteria are grown in presence of labeled methionine which indicates that the methyl group of methionine may become incorporated directly mycolic acids. By the incorporation of either [^{14}C-methyl]- methionine or [^{3}H-methyl]- methionine in growth media and it has been shown that the bridging of methylenes in the cyclopropane ring, the carbon of methoxy functionality and the methyl branches adjacent to trans-olefins, methoxy and keto moieties which are all derived from methionine presumably through S-adenosyl-L-methionine (SAM). Also it long proposed that the meromycolyl cyclopropane groups were derived from the double bonds. [169] The evidence for this idea came with the identification of a gene from *M.tuberculosis* capable of conferring upon *M. smegmatis* , the ability to produce a large amount of cyclopropane containing mycolic acids. [170] This gene is coined CmaA1 for cyclopropane mycolic acid synthase (CMAS) which was found to be 34% identical to the E. coli cyclopropane fatty acid synthase (CFAS) in which the cyclopropane is introduced at the distal position. CmaA1 shows strong homology to other SAM-dependent methyl transferases, particularly in the region known actually to bind SAM. [171]

The corresponding protein based on the sequence of CmaA1 is CmaA2 which was shown to introduce a cyclopropane ring at the proximal position again by characterization of the purified chimeric mycolate obtained following heterologus expression in *M. smegmatis*. This enzyme will cyclopropanate the α-1 but not the α-2 mycolates from *M. smegmatis* suggesting that the enzyme

has a specificity for cis double bonds. However *M.tuberculosis* produces both cis and Tran's cyclopropane containing mycolates, yet CmaA1 and CmaA2 produce only cis-cyclopropanes. Also this suggests the presences of at least one or more enzyme in M.tuberculosis are capable of producing Tran's cyclopropanes. The Trans series of cyclopropanes are always accompanied by an adjacent methyl branch which suggests that the two equivalents of SAM are involved in their formation. The first forms a Tran's olefin with an adjacent methyl group and the second to form the actual cyclopropane ring. [172]

ADME ANALYSIS

For a drug to be pharmacologically active and exert the action it should posses pharmacokinetic properties like absorption, distribution, metabolism and excretion. In the field of drug research and development many drug failures do occur, as they do not undergo these properties satisfactorily. This has to be ruled out earlier in the process of drug discovery. Many in-vitro studies are more frequently used to evaluate ADME properties. Some computational methods (in silico tools) have been evolved to investigate the most suitable drug molecules. [173]

Prediction of ADME related properties[174]

Absorption:

To investigate this in silico models uses simple parameters like log D (diffusion coefficient) and polar surface area are the descriptors foe hydrogen bonding capacity and log P(partition co efficient) values should fall under the prescribed values as per the rule of five, which determines the absorption.

Bioavailability:

Size and shape of the molecule, lipophilicity and flexibility determines the bioavailability.

Metabolism:

Various in silico approaches are existing in evaluating the metabolism namely QSAR and 3D QSAR. Apart from those computational chemists have updated the structural details in the data bases and tools for predicting metabolism.

EVALUATION OF INSILICO TOXICITY[175]

Toxicity is one of the major criteria to be considered for a molecule to shine as a successful clinical candidate in pharmaceutical research. About 20-40 % of drug failure comes under this category. Commercial in silico tools estimates toxicity and provides information by the use of QSAR (parameters and descriptors), scientific literatures and to some extent in abstracting issues from humans.

In silico approaches like OSIRIS property explorer predicts the carcinogenicity, mutagenicity, teratogenicity, immune toxicology, irritation, sensitization, etc. Newly updated tools help in evaluating hepato, neuro and cardio toxicity.

CHARACTERIZATION

IR SPECTROSCOPY [176]

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic chemists. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. IR spectroscopy is an important and popular tool for structural elucidation and compound identification.

The possible characteristic bands of the nucleus are

1. 3300-3540 cm-1 N-H Stretching Vibration
2. 3670-3230 cm-1 O-H Stretching Vibration
3. 1690-1630 cm-1 C=N Stretching Vibration
4. 2975-2840 cm-1 C-H Aliphatic Stretching Vibration
5. 3100-3000 cm-1 C-H Aromatic Stretching Vibration

NMR SPECTROSCOPY [177]

NMR is the most powerful analytical tool currently available to an organic chemist. NMR allows characterization of a very small amount of sample (10mg), and does not destroy the sample (non-destructive technique). NMR spectra can provide vast information about a molecule's structure and can very often be the only way to prove what the compound really is. Typically though, NMR is used in conjunction with other types of spectroscopy and chemical analysis to fully confirm a complicated molecule's structure. It involves the interaction of the electromagnetic radiation and the hydrogen of the nucleus when placed in an external static magnetic field.

Some basic characteristic peaks of the nucleus

1. Aromatic and hetero aromatic compounds 6-8.5 δ
2. Alcoholic hydroxyl protons 1-5.5 δ
3. Aldehyde protons 9-10 δ

MASS SPECTROSCOPY [178]

Mass Spectrometry is an analytic technique that utilizes the degree of deflection of charged particles by a magnetic field to find the relative masses of molecular ions and fragments. It is a powerful method because it provides a great deal of information and can be conducted on tiny samples. Mass spectrometry has a number of applications in organic chemistry. They are:

- ✓ Determining molecular mass
- ✓ Finding out the structure of an unknown substance
- ✓ Verifying the identity and purity of a known substance
- ✓ Providing data on isotopic abundance

BIOLOGICAL EVALUATION

Anti-tubercular Activity

There are various high throughput assays available for screening of new chemical entities against tuberculosis. They are:

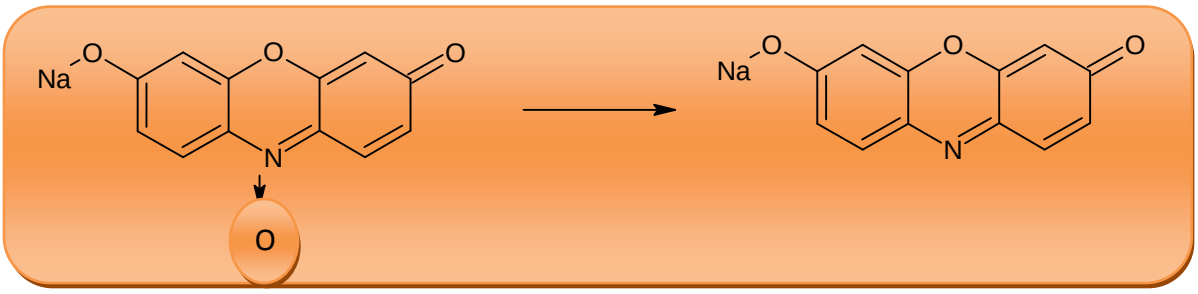
- Microplate Alamar Blue Assay
- BACTEC Assay
- Luciferous Reporter Phage assay
- REMA Assay
- Broth Dilution Assay
- Middle brook(7H 9,7H 10,7H 11) Agar Dilution Assay

THE ALAMAR BLUE ASSAY

Alamar Blue monitors the reducing environment of the living cell. The active ingredient is resazurin (IUPAC name: 7-hydroxy-10-oxidophenoxazin-10-ium-3-one), also known as diazo-resorcinol, azoresorcin, resazoin, resazurine, which is water-soluble, stable in culture medium, is non-toxic and permeable through cell membranes. Continuous monitoring of cells in culture is therefore permitted. [179]

Growth is measured quantitatively by a visual colour change and the amount of fluorescence produced is proportional to the number of the living cells which is determined by colorimetric and fluorimetric methods.

Chemistry [180]



Redox principle

This assay is an indirect colorimetric DST method for determining the MIC of TB drug strains of Mycobacterium tuberculosis. The redox indicator Alamar blue monitors the reducing environment of the living cell. It turns from blue to pink in the presence of mycobacterial growth. As the indicator dye accepts electrons it changes from the blue, oxidized, non fluorescent state to the pink, reduced, fluorescent state. The oxidation-reduction potential of Alamar Blue is +380 mV at pH 7.0, 2□□°C. Alamar Blue, therefore, can be reduced by NADPH (Eo = 320 mV), FADH (Eo = 220 mV), FMNH (Eo = 210 mV), NADH (Eo = 320 mV), as well as the cytochromes (Eo = 290 mV to +80 mV). In addition to mitochondrial reductases, other enzymes (such as the diaphorases (EC 1.8.1.4, dihydrolipoamine dehydrogenase), NAD (P) H: quinine oxidoreductase (EC 1.6.99.2) and flavin reductase (EC 1.6.99.1) located in the cytoplasm and the mitochondria may be able to reduce Alamar Blue. [181]

ADVANTAGES

- It has accurate time course measurement.
- It has high sensitivity and linearity.
- It involves no cell lysis.
- It is ideal for use with post measurement functional assay.
- It is flexible as it can be used with different cell models.
- It is scalable and can be used with fluorescence and/or absorbance based instrumentation platforms.
- It is nontoxic, non-radioactive and is safe for the user.

APPLICATIONS:

- ✓ Especially meant for studies on Mycobacterium tuberculosis.
- ✓ Used extensively in cell viability and cytotoxicity studies. [182]

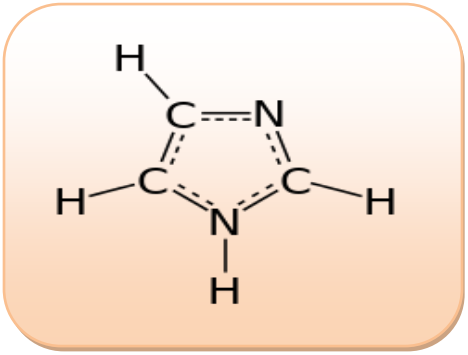
HETEROCYCLIC CHEMISTRY

Heterocyclic structures always are a part in the field of research and development in organic chemistry. Millions of heterocyclic structures are found to exist having special properties and biological importance. Among various compounds, we have chosen imidazole, a fused diazole heterocyclic structure. This ring system is present in important biological building-blocks, such as histidine, and the related hormone histamine. Many drugs contain an imidazole ring. [183]

IMIDAZOLE NUCLEUS:

Imidazole is a planer five-member heterocyclic ring with 3C and 2N atom and in ring N is present in 1st and 3rd positions. The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine and nucleic acid. Being a polar and ionisable aromatic compound, it improves pharmacokinetic characteristics of lead molecules and thus used as a remedy to optimize solubility and bioavailability parameters of proposed poorly soluble lead molecules. [184] Imidazole derivatives have occupied a unique place in the field of medicinal chemistry. The incorporation of the imidazole nucleus is an important synthetic strategy in drug discovery. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents. Imidazole drugs have broadened scope in remedying various dispositions in clinical medicines. Numerous methods for the synthesis of imidazole and also their various structure reactions offer enormous scope in the field of medicinal chemistry. [185]

1*H*-Imidazole



On the basis of various literature surveys Imidazole derivatives shows various pharmacological activities [187,188]

- Anti-tubercular activity[186]
- Anti-fungal and Anti-bacterial activity

- Anti-inflammatory activity and analgesic activity
- Anti-depressant activity
- Anti-cancer activity[189]
- Anti-viral activity
- Antileishmanial activity

On view of the importance of the imidazole nucleus. It was decided to design nucleus based on the imidazole nucleus. One hundred different molecules with the imidazole scaffold were drawn and docked.

Aim and plan of work

Research Envisaged and Plan of Work

Objective of the Present Study

AIM

The literature survey reveals to develop novel and potent cyclopropane mycolic acid synthase – 2 (cmaA – 2) inhibitors with anti-tubercular activity.

OBJECTIVE

- Identification of the common Pharmacophore responsible for the inhibition of cmaA – 2 using Hiphop module of Catalyst® software 4.11 from Accelrys.
- Using scaffold hopping technique, generation of 10,000 scaffolds from the drug.
- Prediction of anti-tubercular activity for the designed using the Hypo refine model and to identify novel and potent cmaA – 2 inhibitor using Lipinski rule of five.
- The potent cmaA – 2 inhibitors attained as results can be used as lead for drug development.
- Docking of the lead with various derivatives using Glide software to the target 1KPI.
- The derivatives of the compounds from the lead molecule which has higher Glide score value were synthesized.
- Synthesis and characterization

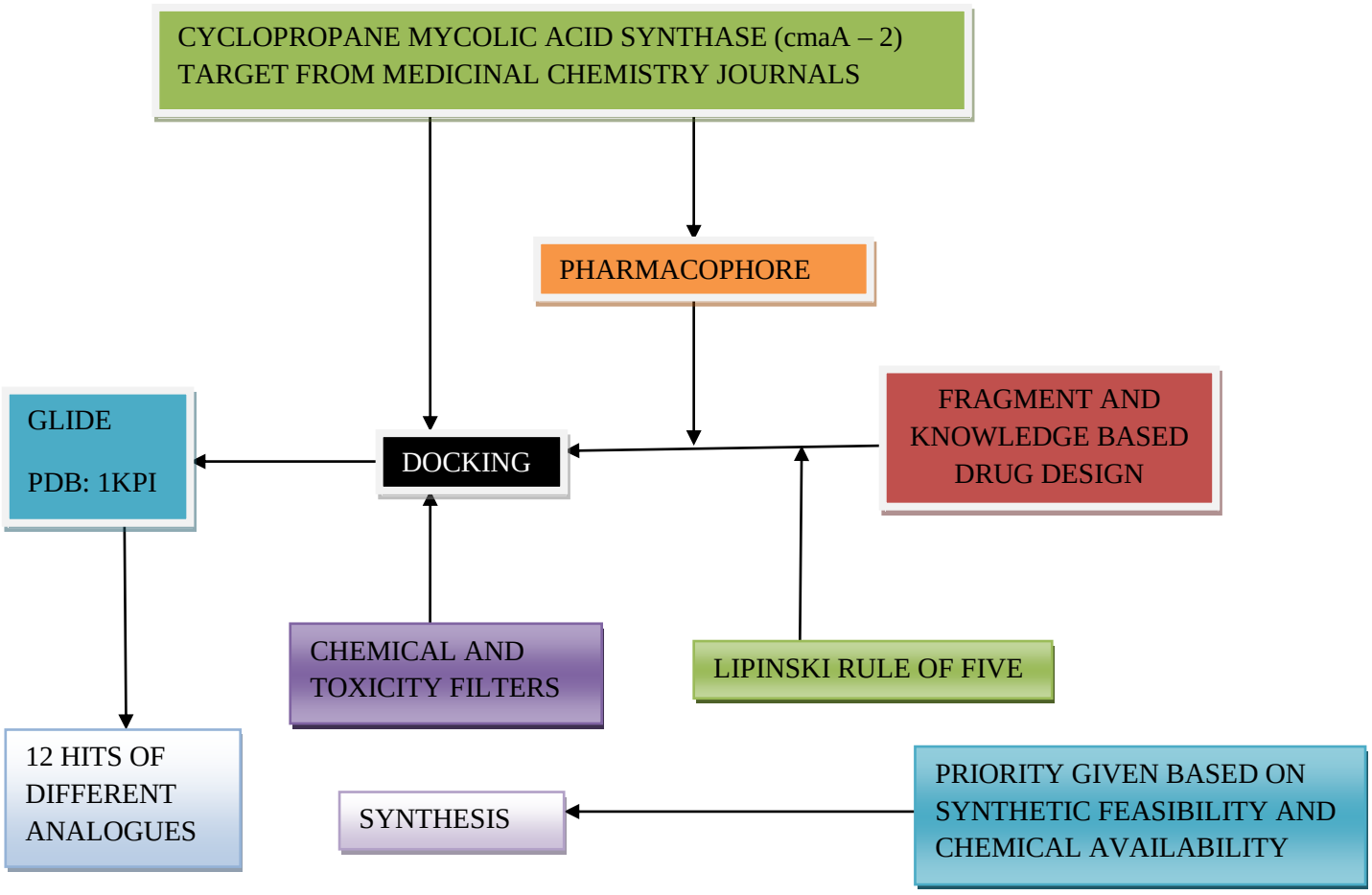
    - ✓ Synthesis of substituted imidazole.
    - ✓ Synthesis of title compounds
    - ✓ Study of physical properties of the synthesized compounds
        - Melting point, Percentage yield.
        - TLC profile.
        - Solubility.

✓ Spectral studies

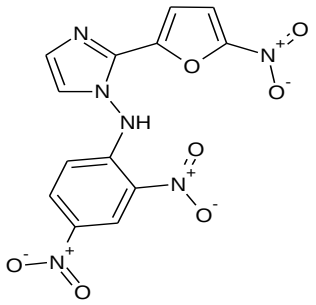
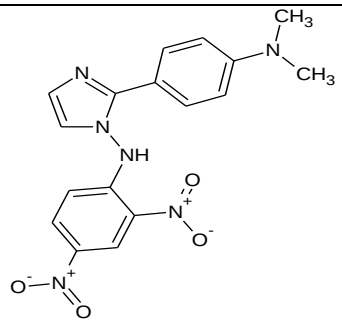
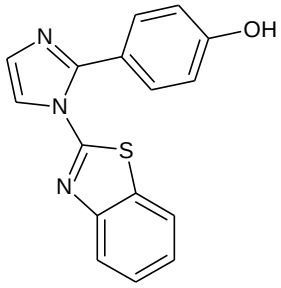
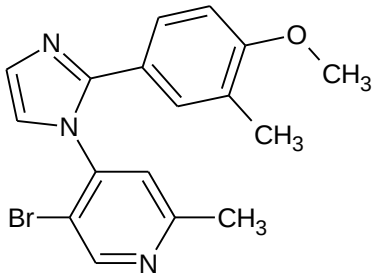
The chemical structures of the synthesized compounds were characterized by means of IR, ^{1}H-NMR, ^{13}C-NMR and MASS spectral analysis.

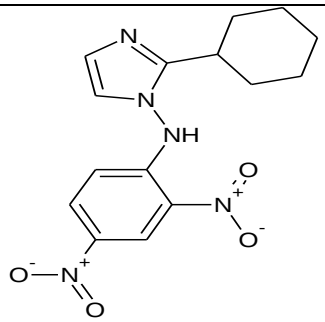
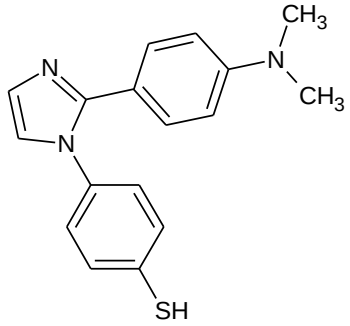
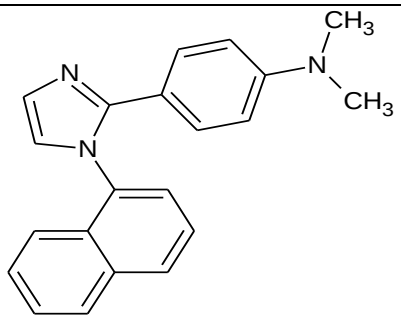
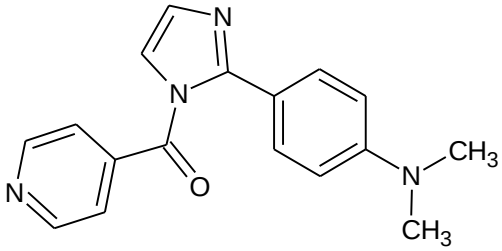
The present study carried out based on the below flow

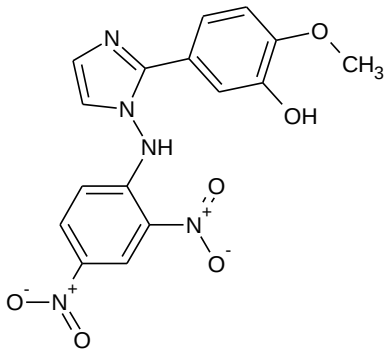
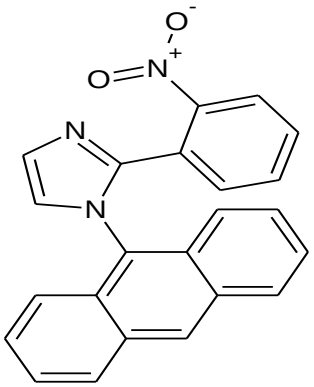
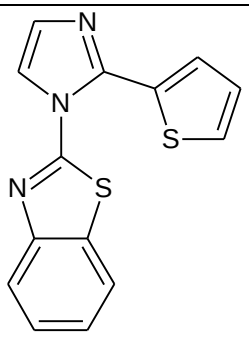
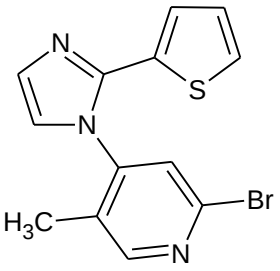
.



List of compounds to be synthesized

ENTRY	COMPOUNDS	IUPAC
SU_1		*N*-(2,4-dinitrophenyl)-2-(5-nitrofuran-2-yl)-1*H*-imidazol-1-amine
SU_2		2-[4-(dimethylamino)phenyl]-*N*-(2,4-dinitrophenyl)-1*H*-imidazol-1-amine
SU_3		4-[1-(1,3-benzothiazol-2-yl)-1*H*-imidazol-2-yl]phenol .
SU_4		5-bromo-4-[2-(4-methoxy-3-methylphenyl)-1*H*-imidazol-1-yl]-2-methylpyridine .

SU_5		2-cyclohexyl-*N*-(2,4-dinitrophenyl)-1*H*-imidazol-1-amine .
SU_6		4-{2-[4-(dimethylamino)phenyl]-1*H*-imidazol-1-yl}benzenethiol .
SU_7		*N*,*N*-dimethyl-4-[1-(naphthalen-1-yl)-1*H*-imidazol-2-yl]aniline .
SU_8		*N*-{2-[4-(dimethylamino)phenyl]-1*H*-imidazol-1-yl}pyridine-4-carboxamide .

SU_9		5-{1-[(2,4-dinitrophenyl)amino]-1*H*-imidazol-2-yl}-2-methoxyphenol
SU_{10}		1-(anthracen-9-yl)-2-(2-nitrophenyl)-1*H*-imidazole .
SU_{11}		2-[2-(thiophen-2-yl)-1*H*-imidazol-1-yl]-1,3-benzothiazole .
SU_{12}		2-bromo-5-methyl-4-[2-(thiophen-2-yl)-1*H*-imidazol-1-yl]pyridine

Review of Literature

1. Rahul Jain *et al.,(2005)* Tuberculosis (TB) is one of the most devastating diseases primarily due to several decades of neglect,and presents a global health threat of escalating proportions. TB is the second leading infectious causes ofmortality today behind only HIV/AIDS.

2. **George KM,*et al.,(2008)*** The biosynthesis of cyclopropanated mycolic acids in Mycobacterium tuberculosis. Identification and functional analysis of CMAS-2 revealed the gene whose product cyclopropanates the proximal double bond was cloned by homology to a putative cyclopropane synthase identified from the Mycobacterium leprae genome sequencing project. This gene, named cma2, was sequenced and found to be 52% identical to cma1 (which cyclopropanates the distal double bond) and 73% identical to the gene from M. leprae. Both cma genes were found to be restricted in distribution to pathogenic species of mycobacteria. Expression of cma2 in Mycobacterium smegmatis resulted in the cyclopropanation of the proximal double bond in the alpha 1 series of mycolic acids.

3 .Dominique Guianvarc'h,*et al.,(2009)* Identification of inhibitors of the E. coli cyclopropane fatty acid synthase from the screening of a chemical library: revealed an in vitro and in vivo studies, using an automated coupled colorimetric assay for the Escherichia coli cyclopropane fatty acid synthase (CFAS).

4. Christine *et al.(2007),* Synthesis and evaluation of analogues of S-adenosyl-L-methionine, as inhibitors of the E. coli cyclopropane fatty acid synthase. They synthesised some analogues of S-adenosyl-l-methionine were synthesized and evaluated as inhibitors of the purified E. coli cyclopropane fatty acid synthase, a model for M. tuberculosis cyclopropane synthases that are potential targets for antituberculous drugs.

5. Cécile Asselineau *et al.(2003),* reviewed the biosynthesis of mycolic acids by mycobacteria: current and alternative hypotheses by adding the classical elongation process of fatty acid synthesis produces two long chains, the condensation of which leads to the direct precursors of mycolic acids.

6. Michael S. Glickman *et al.,(2012)* revealed that Mycobacterium tuberculosis lacking all mycolic acid cyclopropanation is viable but highly attenuated and hyper inflammatory in mice.

7. James C. Sacchettini *et al.,(2004)* worked on TB drug discovery: addressing issues of persistence and resistance by reviewing the recent developments of some of the pathways involved in a persistent infection and pathogenesis of mycobacterium tuberculosis, which reveal new targets for drug development.

8. **Liao RZ *et al,(1978)*** Mechanism of mycolic acid cyclopropane synthase: a theoretical study. They demonstrated that the reaction starts via the transfer of a methyl to the substrate double bond, followed by the transfer of a proton from the methyl cation to the bicarbonate present in the active site.

9. Chih-chin Huang *et al*.(2012), Mycolic acids are major components of the cell wall of *Mycobacterium tuberculosis*. Several studies indicate that functional groups in the acyl chain of mycolic acids are important for pathogenesis and persistence. There are at least three mycolic acid cyclopropane synthases (PcaA, CmaA1, and CmaA2) that areresponsible for these site-specific modifications of mycolic acids.

10. Romano T. Kroemeret *et al.(2003),* this review gives an introduction into ligand – receptor docking and illustrates the basic underlying concepts. An overview of different approaches and algorithms are provided. Although the application of docking and scoring has led to some remarkable successes, there are still some major challenges ahead, which are outlined here as well. Approaches to address some of these challenges and the latest developments in the area are presented.

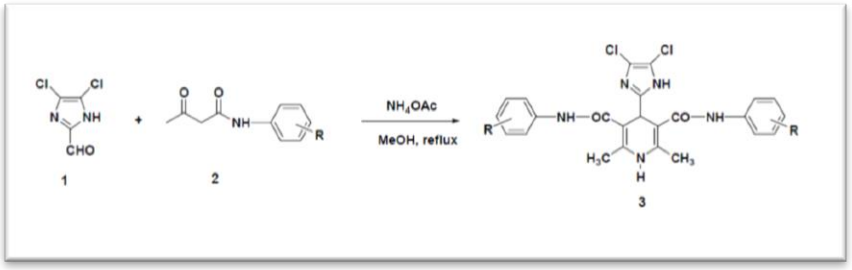
11.Thomas Hughes,*et al.(2009),* It is the mission of pharmaceutical research companies to take the path from understanding a disease to bringing a safe and effective new treatment to

patients. Scientists work to piece together the basiccauses of disease at the level of genes, proteins and cells.

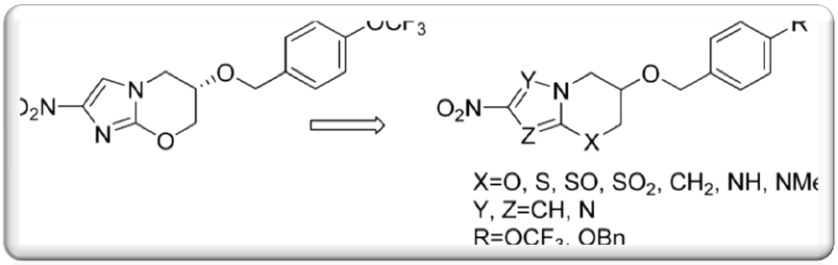
12. Andrew Worth *et al.(1998),* Distribution, Metabolism and Excretion (ADME) properties, which are often important in discriminating between the toxicological profiles of parent compounds and their metabolites/degradation products. The review was performed in a broad sense, with emphasis on QSARs and rule-based approaches and their applicability to estimation of oral bioavailability, human intestinal absorption, blood-brain barrier penetration, plasma protein

binding, metabolism and. This revealed a vast and rapidly growing literature and a range of software tools.

13. Shafiee A.*et al.,(2008)* Synthesis and antitubercular activity of new N,N-diaryl-4-(4,5-dichloroimidazole-2-yl)-1,4-dihydro2,6dimethyl3,5pyridinedicarboxamides.Dihydropyridines having carboxamides in 3 and 5 positions show anti-tuberculosis activity. The purpose of the present study was tosynthesize new DHPs having possible anti-tuberculosis activity.

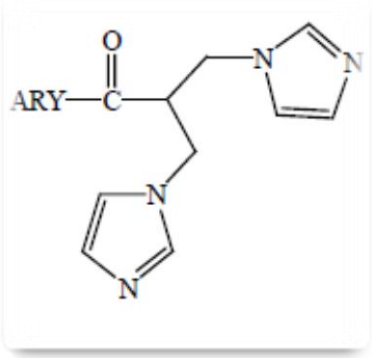


14.Thompson,*et al.,(1995)* Synthesis, Reduction Potentials, and Antitubercular Activity of Ring A/B Analogues of the Bioreductive Drug (6*S*)-2-Nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5*H*-imidazo[2,1*b*][1,3]oxazine (PA-824)

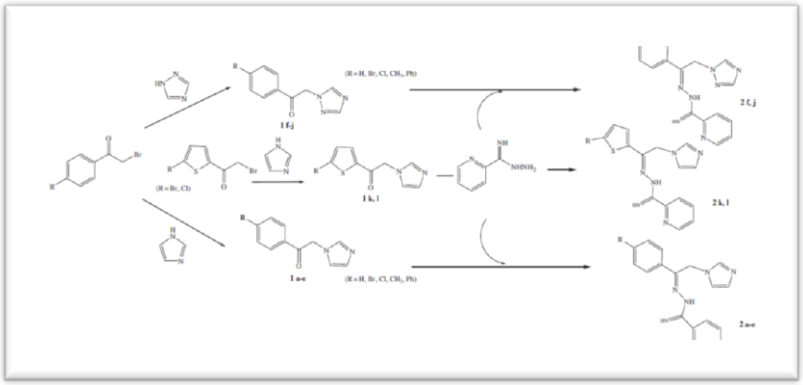


15. Rawat M.S.M.*et al*.,(2005) *The 5-nitroimidazole is an important class of imidazole based drugs. The 5-nitroimidazoles are a well-established group of protozoal and bactericidal agents but after discovery of imidazole drugs many protozoa and bacteria has developed resistance towards drugs in market.*

16. Daniele Zampieriet *et al*.(2003), Synthesis, antifungal and antimycobacterial activities of new bisimidazolederivatives.

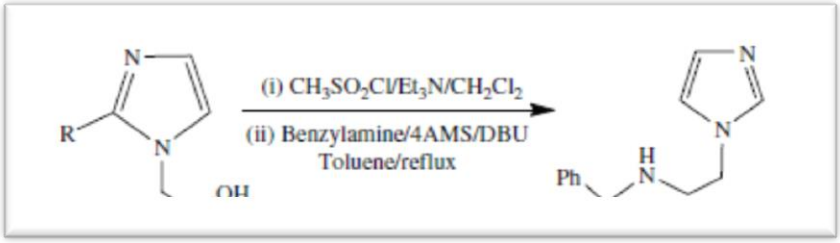


17. Elena Banfi *et al*.,(1998) Antifungal and antimycobacterial activity of new imidazole and triazole derivatives. A combined experimental andcomputational approach.

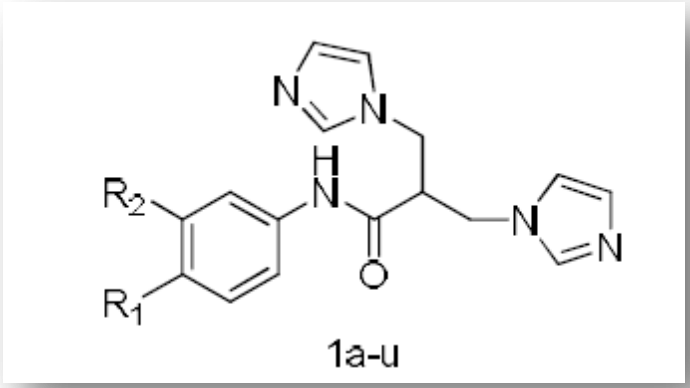


18.Rama P. Tripathi *et al*.,(2013) A series of imidazole based compounds were synthesized by reacting simple imidazoles with alkyl halides or alkyl halocarboxylate in presence of tetrabutylammonium bromide (TBAB). The compounds bearing carbethoxy group undergo amidation with different amines in the presence of DBU to give respective carboxamides. The synthesized compounds were screened against Mycobacterium tuberculosis where compound 17 exhibited very good in vitro antitubercular activity and may serve as a lead for

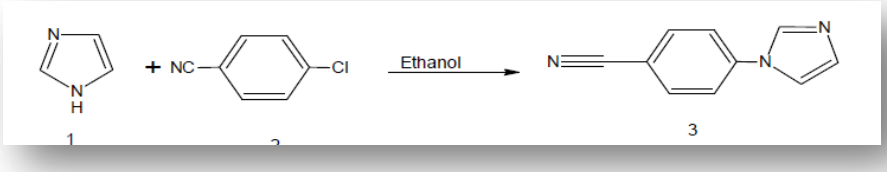
further optimization.



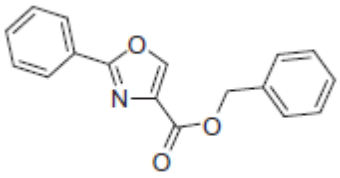
19. Prabh Simran Singh *et al*.,(2012) study of imidazole derivatives to identify them asmycobacterium tuberculosis 14dm inhibitors.



20. **Prasanthy G *et al*., (2006** a new series of 1-substituted imidazole derivatives were synthesized taking different anilines and sulfonamides as substitutions. The chemical structures were confirmed by means of IR, 1H-NMR and Mass spectral data. The compounds were screened for their anticancer and antimicrobial activities. N-(3-chloro-4-fluorophenyl)-4-(1H-imidazol-1-yl)benzamide exhibited highest activity against cervical cancer. 4-(1H-imidazol-1-yl)-N-(4-(N-(5-methylisoxazol-4-yl) sulfamoyl) phenyl) benzamide showed good antifungal activity. 4-(1H-imidazol-1-yl)-N-(4-(N-thiazol-4-ylsulfamoyl) phenyl) benzamide showed good antibacterial activity.

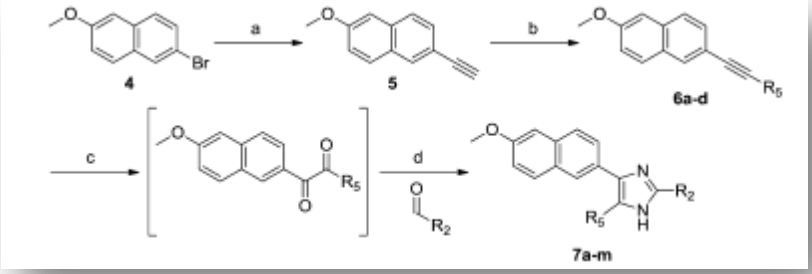


21.Eufrânio N. da Silva Jr. *et al*.,(2007) synthesis of aryl substituted imidazoles and oxazoles and their potent activity against mycobacteriumtuberculosis.

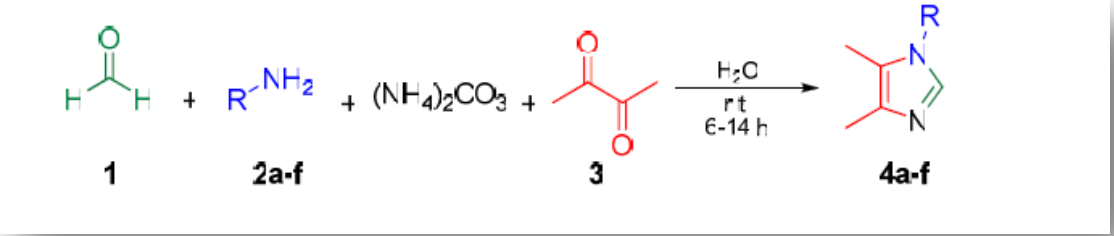


22.Gupta P,*et al*.,(2001) We describe in vitro anti-Mycobacterium tuberculosis activities of ring-substituted-1H-imidazole-4- carboxylic acid derivatives (1-6), and 3-(2-alkyl-1H-imidazol-4-yl)-propionic acid derivatives (7-13)against drug-sensitive and drug-resistant M. tuberculosis H37Rv strains.

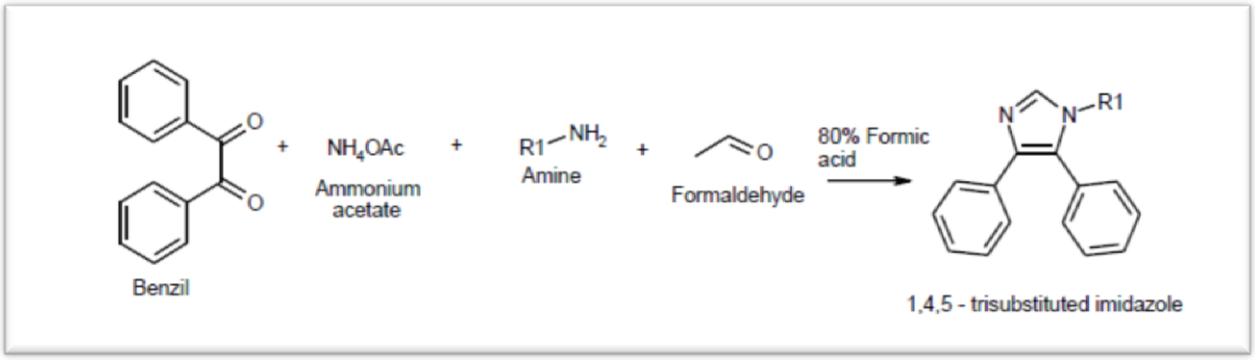
23.Johan Gising *et al*.(2008), Trisubstituted Imidazoles as Mycobacterium tuberculosis Glutamine Synthetase Inhibitors.



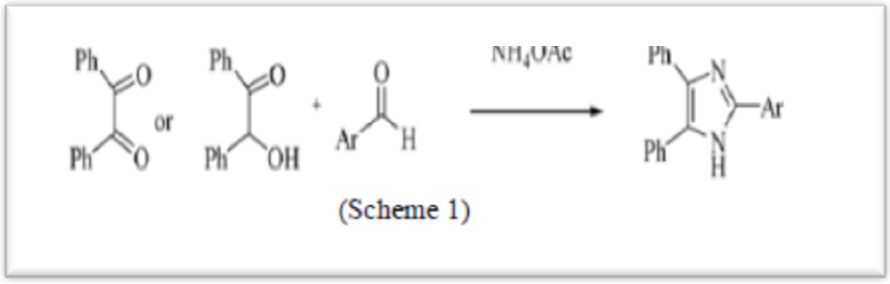
24.Flavia M. da Silva *et al*.,(1988) a new, simple and eco-friendly protocol has been developed for the preparation of trisubstituted imidazoles through the radziszewski reaction between formaldehyde (37%aqueous solution), amines, ammonium carbonate and biacetyl.



25. **Harsha Tripathy *et al*.(2003),** The reactants, 0.210g (1 millimole) of benzil, 0.076g(1 millimole) of ammonium acetate, 0.050g(1 millimole) of paraformaldehyde, 2-3 drops of 80% formic acid and 2 millimoles of amine were taken in 1 mL dry DMF in a long necked glass vial. The reaction mixture was irradiated in synthetic microwave oven (Emry's optimizer). The reaction conditions were optimized in Emry's optimizer as Irradiation time: 8 minutes, Temperature: 120 °C, Pre-stirring time: 60 seconds, Absorption: Normal, Pressure: 19 bars.

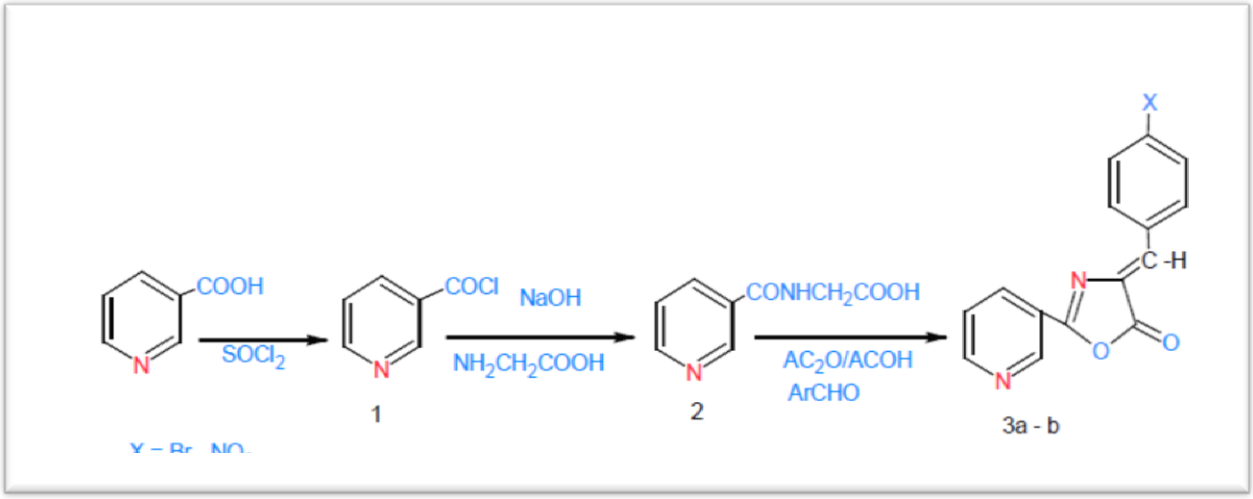


26. **Reza Tayebee *et al*.,(1985)** The new results concerning synthesis of some 2,4,5-triaryl-1H-imidazoles in the absence of any additive, as catalyst, is presented. Moreover, we modified the experimental route for the isolation and purification of the un-reacted benzyl, as initial reactant, from products at the end of the reaction.

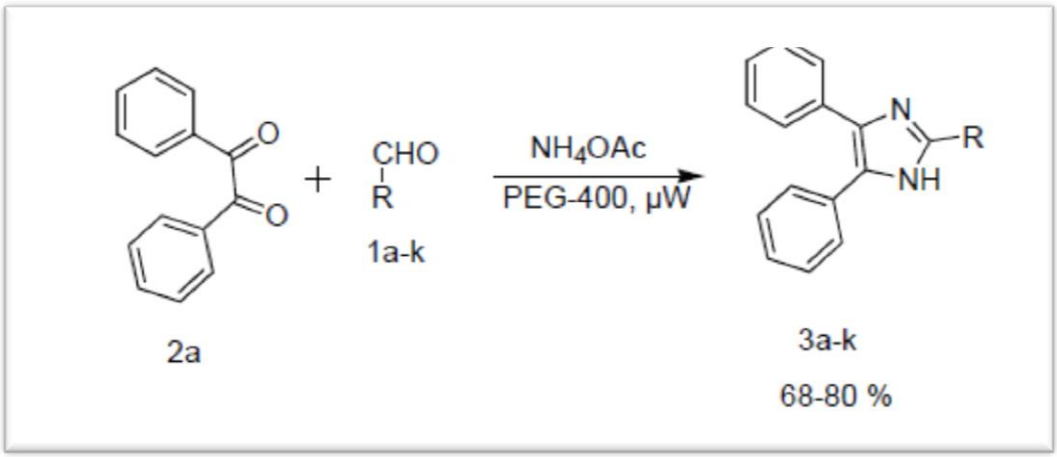


(Scheme 1)

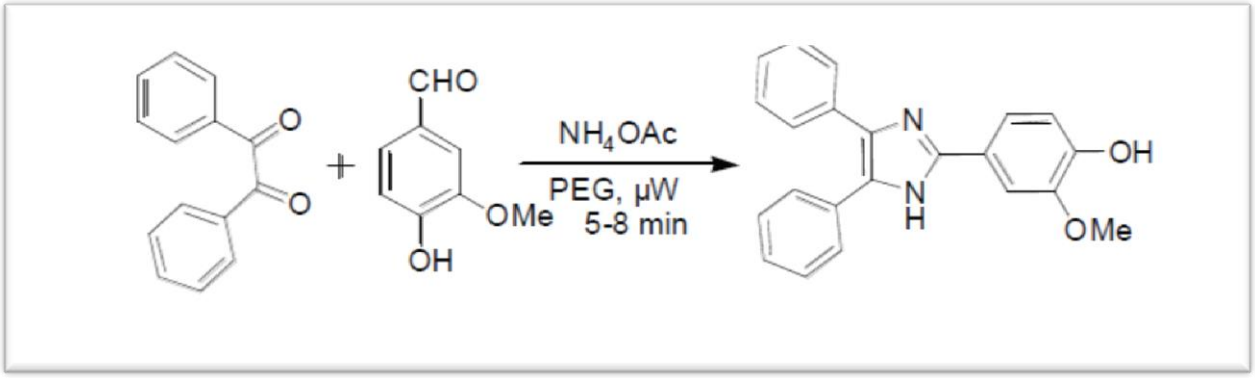
27.**AL. Abodi *et al*.,(1988)** New 1,3-oxazol-5(4*H*)-one (3a – b) was synthesized by cyclization of[(Pyridyl-3-yl-carbonyl)amino]acetic acid (2). The starting were readily obtained by acylation of 2-amino acetic acid (Glycine) with nicotinoyl chloride. Imidazole was synthesized by reaction of compounds (3a - b) with hydrazine hydrate (99%). Compounds (4a - b) were converted into a variety of derivatives. All new compounds were characterized by 1H NMR and FTIR spectroscopy.



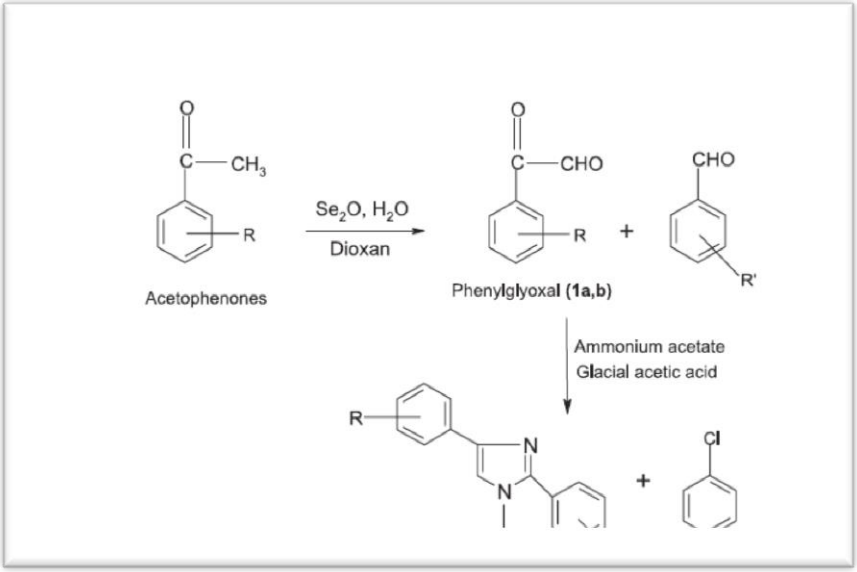
28.Rajendra P. Pawar *et al*.*(2010),*** An efficient and green procedure for the synthesis of 2, 4, 6-triaryl-1H-imidazole in polyethylene glycol under microwave irradiation in excellent yield has been developed. Polyethylene glycol is non toxic, reusable, inexpensive andeasily available.



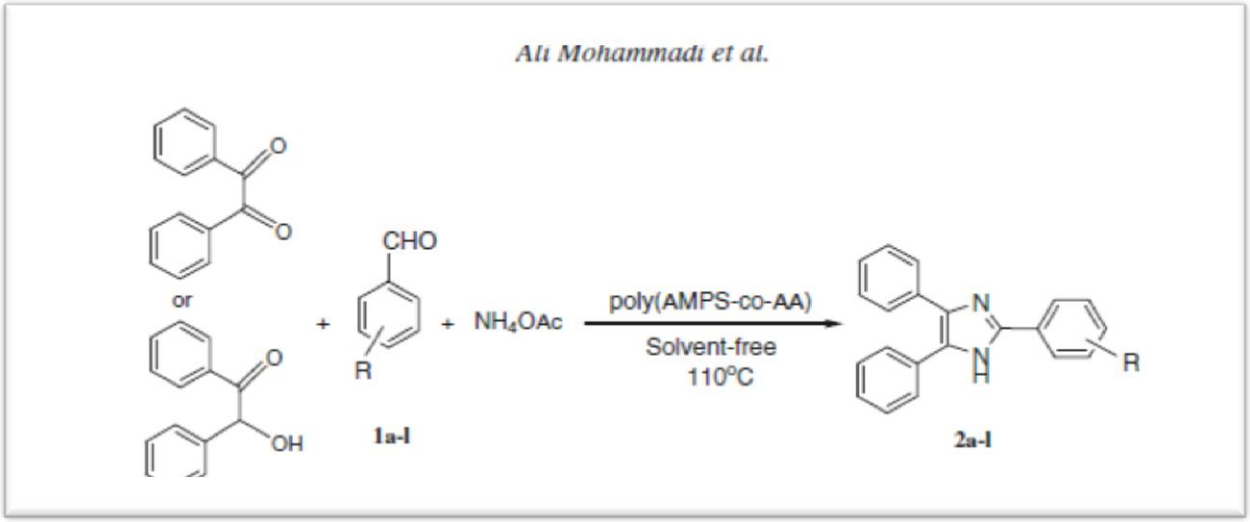
29. Sidhanath V. Bhosale *et al*.,(2007) A series of 2,4,5-triaryl-1H-imidazole was synthesized in one step. We demonstrated an efficient protocol for the synthesis of substituted imidazole from benzil/benzoin, aldehydes and ammonium acetate without catalyst in polyethylene glycol. Product isolation *via* filtration without any hazardous organic solvent claims an environmentally benign protocol for the synthesis of substituted imidazoles.



30. ASIF HUSAIN *et al*.,(2005) Disubstituted imidazoles were prepared by reacting appropriate phenylglyoxal with different aryl aldehydes in the presence of ammonium acetate. Trisubstituted imidazoles were prepared by reacting disubstituted imidazoles with chlorobenzene in the presence of catalytic amount of triethylamine (TEA). The synthesized compounds were characterized on the basis of IR, 1H-NMR and mass spectral data and elemental analysis results. They were tested for their antiinflammatory and antimicrobial actions.

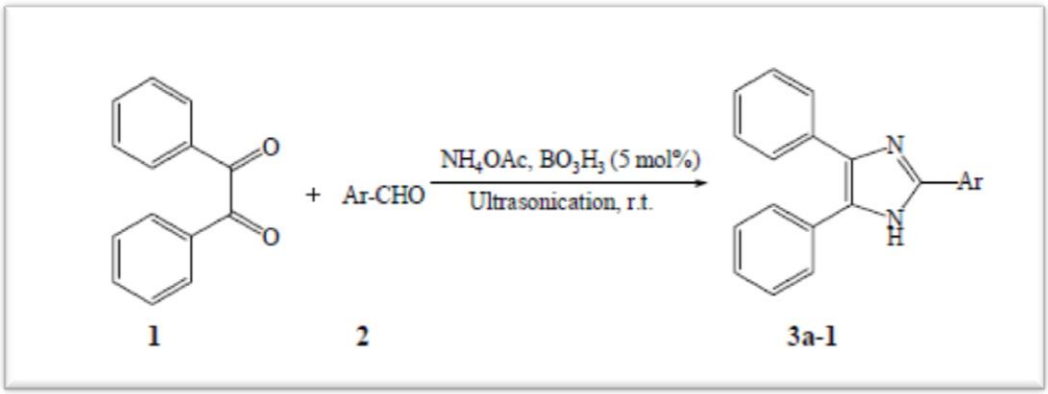


31.ALI MOHAMMADI *et al*.,92003) An efficient synthesis of 2,4,5-trisubstituted imidazoles is achieved by three component cyclocondensation of benzil or benzoin, aldehyde and ammonium acetate by using novel polymeric catalyst [poly(AMPS-co-AA)] under solvent-free conditions. The key advantages of this process are high yields, shorter reaction times, easy work-up, purification of products by non-chromatographic method and the reusability of the catalyst.

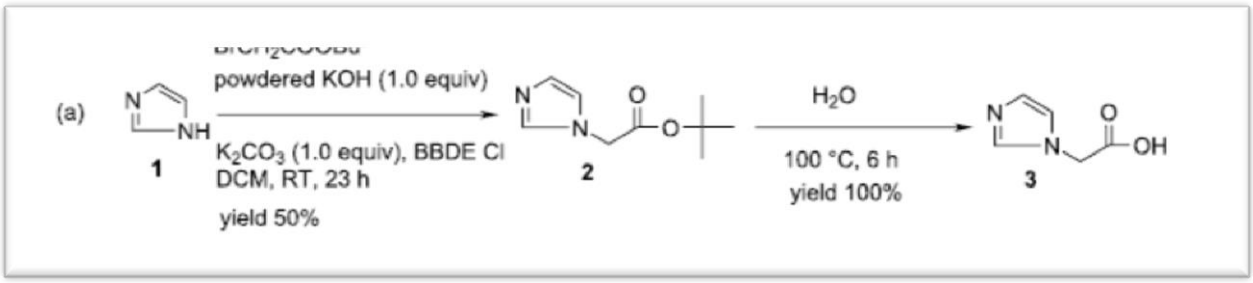


32. **Jadwiga Handzlik *et al*.,(2001)** The paper focuses on recent achievements in the search for new chemical compounds able to inhibit multidrug resistance (MDR) mechanisms in Gram-positive pathogens. An analysis of the results of the search for new efflux pump inhibitors (EPIs) for Gram-positive bacteria, which have been performed over the last decade, indicates that almost all efforts are focused on the NorA (MFS) efflux pump in *S. aureus*

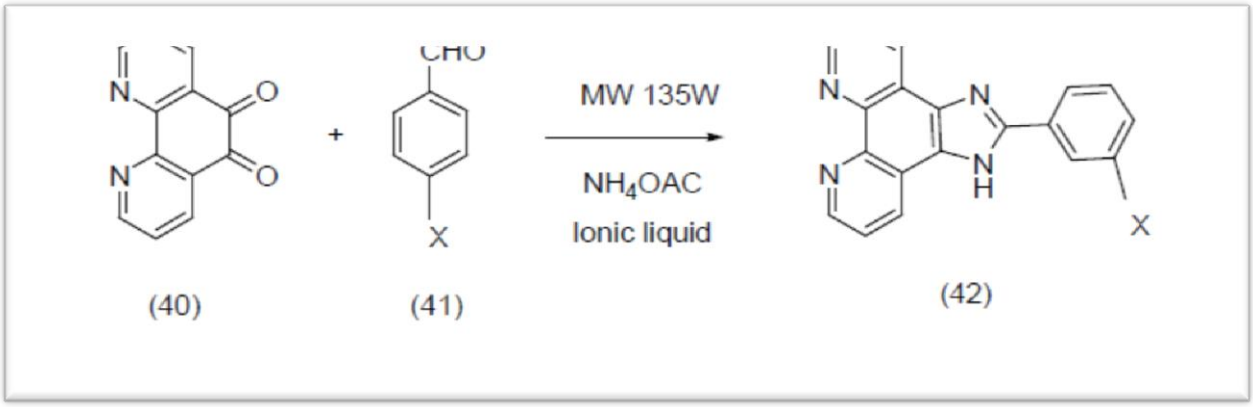
33.Murlidhar S. Shingare *et al*.,(1997) Boric acid (BO3H3) is an inexpensive, efficient and mild catalyst for the synthesis of 2,4,5-triaryl-1*H*-imidazoles in excellent yields from the one-pot three-component condensation of benzil/benzoin, an aldehydes and ammonium acetate in aqueous media under ultrasound at room temperature. The remarkable advantages offered by this method are green catalyst, mild reaction conditions, simple procedures, much faster reactions and excellent yield of products.



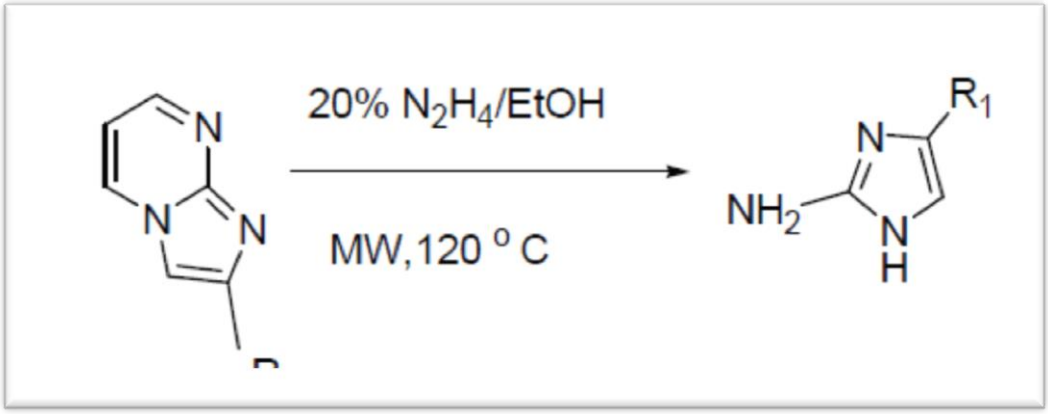
34.Santosh Kumar Singh *et al.*,(1999) A convenient and practical synthesis of imidazol-1-yl-acetic acid hydrochloride was achieved via N-alkylation of imidazole using *tert*-butyl chloroacetate followed by a non-aqueous ester cleavage of the resulting imidazol-1-yl-acetic acid *tert*-butyl ester in the presence of titanium tetrachloride. The synthesized imidazol-1-yl-acetic acid hydrochloride was then utilized to prepare zoledronic acid.



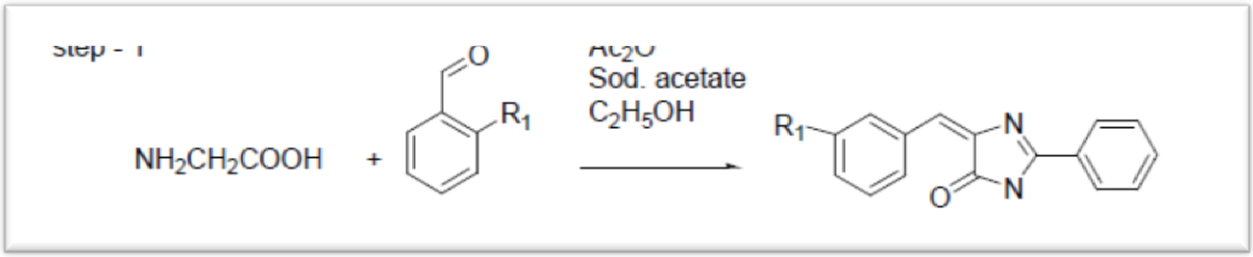
35. Qasim *et al.,(2009)*synthesized 2- phenylimidazo [4,5-f] [1,10] Phenanthroline derivatives (42), by reacting dicarbonyl compound (40) and *p*-substituted benzaldehyde (41), this is a type of acid catalyzed reaction with excellent yields in a neutral ionic liquid, 1-methyl-3-heptylimidazolium tetrafluoroborate [(HeMIM) BF4], under solvent free and microwave assisted conditions. This particular reaction accompanies all the merits of microwave reactions like easy workup, better yield, and environment friendly reaction



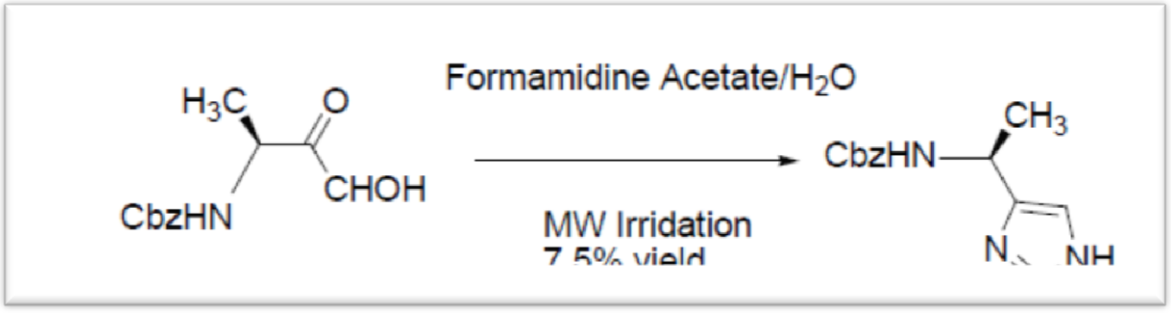
36.Ermolat *et al.,(2006)* synthesized mono and disubstituted-2-amino-1H imidazoles via microwave assisted hydrazinolysis of substituted imidazo [1, 2 a] pyrimidines is reported.This method avoids strong acidic conditions and is superior to the conventional cyclocondensation of a haloketones with N-acetyl guanidine.



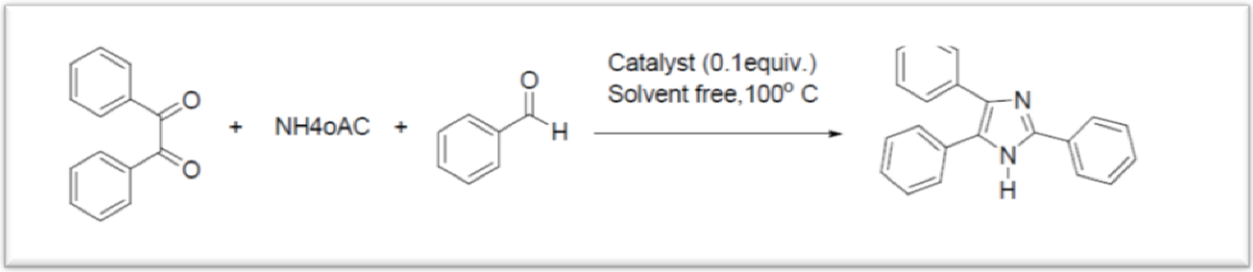
37. Bharadwaj *et al* .,(2010) performed the condensation of different oxazolones (1a-f) (51) with 5- (4-nitrophenyl)-1, 3, 4-thiadiazol-2-amin under microwave oven. The structures of the synthesized compounds , 3a-3j were confirmed on the basis of spectral and elemental analysis. The synthesised compounds were found in better yield than in conventional methods and also screened for *in vitro* antimicrobial study.



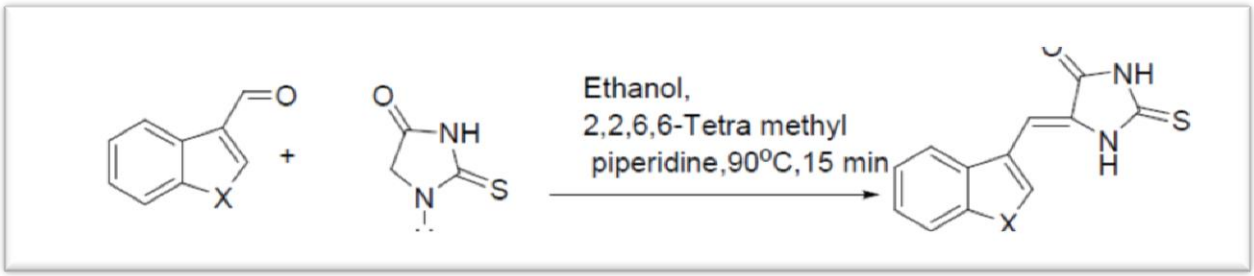
38.Marek *et al* (2007) synthesized via a facile 4-step reaction sequence starting from commercially available and inexpensive N-Cbz amino acids . The condensation of the corresponding α bromoketones with formamidine acetate in liquid ammonia was revealed to be a useful method for the synthesis of such imidazole derivatives , derivatives thus prepared are structurallyrelatedto histamine.



39. Safari *et al* .,(1989) (NH4)6.Mo.7O2. 4H2O was used as an efficient catalyst for an improved and rapid synthesis of 2,4,5-trisubstituted imidazoles by a three-component, one-pot condensation of benzil , aryl aldehydes and ammonium acetate in good yields under solvent-free conditions using microwave irradiation. The reactions in conventional heatingconditions were compared with the microwave-assisted reactions.

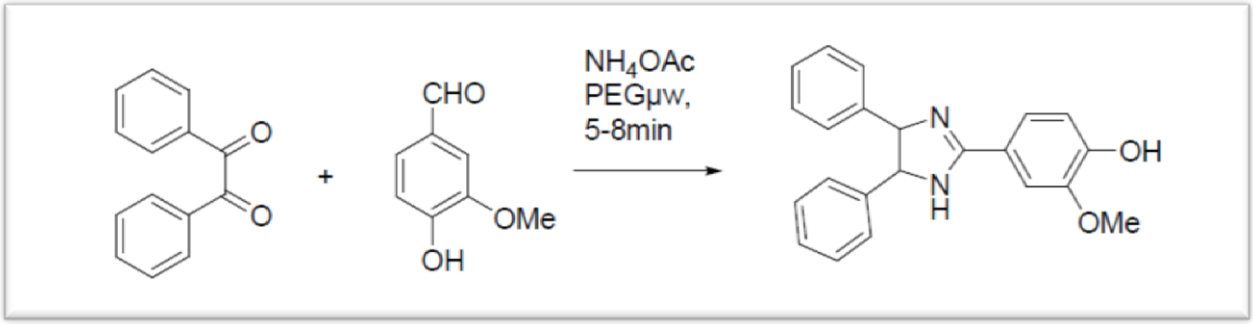


40. Kamila *et al.,(2003)* 2-(Alkyl-1-yl)-1H-imidazol-5(4H)-ones were synthesized *via* nucleophilic substitution of the methylsulfanyl group of the corresponding 2-(methylthio)-1Himidazol- 5(4H)-ones 3a–c with suitably substituted secondary amines . The starting 2-thioxo- imidazolidin-4-ones 2a, 2b were prepared by condensation of thiohydantoin and benzo[b]- thiophene-3-carbaldehyde or benzofuran-3-carbaldehyde under microwave irradiation (MW) conditions. 2-Methylthio derivatives 3a–c were prepared by treatment of 2a–b with methyl iodide in the presence of aqueous sodium hydroxide

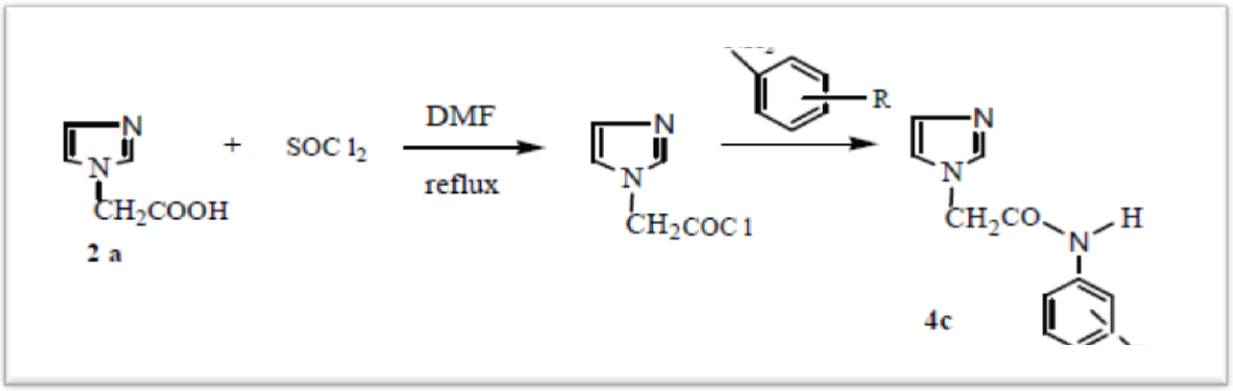


41.Nalage *et al* .,(2012)described an efficient and green procedure for the synthesis of 2, 4, 6-triaryl- 1H-imidazole in polyethylene glycol by condensing benzil and 3-methoxy- 4-hydroxyl benzaldehyde under microwave irradiation in excellent yield has been developed.Polyethylene glycol is non toxic, reusable, inexpensive and easily available.

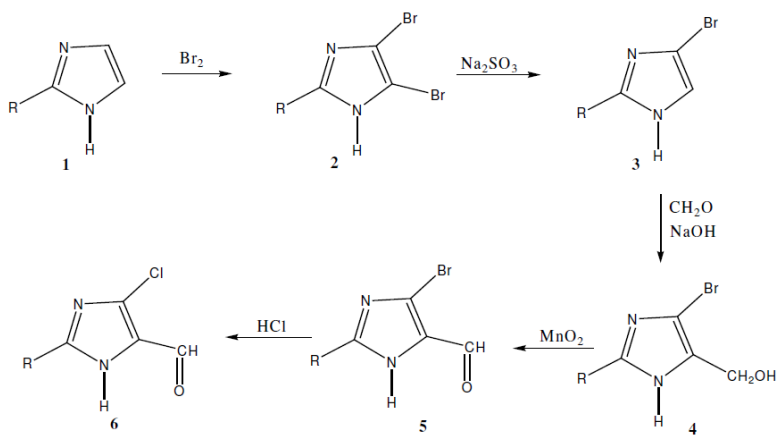
.



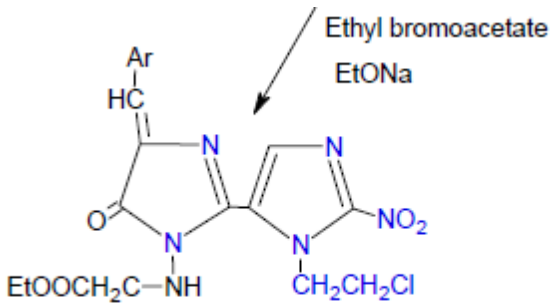
42.A.P. Rajput *et al*.,(2001) 1*H*-Derivatives of azoles like imidazole-1-acetic acid, benzimidazole-1-acetic acid [1-2] benzotriazole-1-acetic acid [3-4], piperazine-1-acetic acid have received very little attention. Literature survey finds very little information about these compounds. Interest in these azole containing structure stem.



43.Abbas SHAFIEE *et al*.(2012) 4(5)-Chloro-imidazole-5(4)-carboxaldehyde derivatives are important precursors for the preparation of biologically active compounds. We developed a simple, novel, and efficient method for the synthesis of these compounds. The chemistry described is amenable to large-scale use and is flexible enough to allow the preparation of analogs.



44. Abdul Jabar Kh. Atia *et al*.,(1996) Metronidazole (MTZ, **1**) is a synthetic compound used in the treatment of infections caused by Gram negative anaerobic bacteria like *Helicobacter pylori* and protozoa such as *Giardia*, *Lamblia*, and *Entomoeba histolytica*, Imidazole and its derivatives are of great significance due to their important roles in biological systems, particularly in enzymes, as proton donors and/or acceptors, coordination system ligands and the base of charge–transfer processes.



9 a - c

45. Maria Grazia Mamolo *et al*., 2009 new bis-imidazole derivatives have been synthesized and their antifungal and antimycobacterial activity was determined.Almost all compounds exhibited a moderate to good activity against two clinical isolates of Candida albicans 3038 and Candida glabrata123. The same compounds showed an interesting killing activity against Mycobacterium tuberculosis H37Rv reference strain.

Materials and methods

DOCKING STUDIES (GLIDE)

DOCKING AND SCORING METHODS:

Docking procedure aims to identify the correct binding poses within the binding site of the protein while the scoring function aims to predict binding affinity of ligand for the protein binding region [190]. The scoring function serves three purposes.

1. For ranking the conformations generated by the docking, search for one ligand interacting with a given protein, this aspect is essential to detect the best binding site mode.
2. For ranking different ligands with respect to binding to one protein i.e. prioritizing ligands according to their affinity, this aspect is essential in virtual screening.
3. For ranking one or different ligands with respect to their binding affinity to different proteins their aspect is essential for the consideration of specificity and selectivity.

STEPS IN DOCKING:

PROTEIN PREPARATION [191,192,193]

A typical PDB file downloaded from protein data bank consists of heavy metals, can contain waters, cofactors, metal ions and can be dimeric or multimeric. The structure generally has no information on bond orders, topologies, or formal atomic charges. The Terminal groups can also be misaligned because the X-ray structure analysis cannot easily distinguish between O and NH_2 ion. The ionization and tautomeric states are usually unassigned. Generally Glide calculations use an all atom force-field for accurate energy evaluation and thus, Glide requires bond orders and ionization states which to be properly assigned and should performs better when side chains are reoriented, when necessary and steric clashes are relieved.

The steps for the protein preparation carried out are as follows:

1. The ligand/protein co-crystallized structure of CmaA-2 target (1KPI) in the form of PDB was imported into maestro. The preparation component of a protein preparation facility requires an identified ligand which to be verified.
2. The protein-ligand complex is then identified for its form as dimer or other multimer, containing duplicate binding sites, and other duplicate chains that are redundant. Then

remove the redundant binding sites and the associated chains by picking and deleting molecules or chains.

3. The waters to be identified that bridge between the ligand and protein are retained and all the other waters (except those coordinated to metals) are deleted and if waters are added then hydrogen can be automatically added and then the orientations of water molecules are checked once again.
4. The protein, metal ions and cofactors are then adjusted. The structures that are missing residues near the active sites should be repaired. The covalent bonds from the metal ions to the protein should be changed to zero-order bonds. The formal charges on the metal and the ligating groups should be adjusted to their appropriate values.
5. The ligand bond orders and the formal charges are adjusted. In Glide models such interactions are associated with vanderwaals and electrostatic interactions.
6. The restrained minimization of protein structure reorients the side chain containing hydroxyl groups and alleviates the potential steric clashes. The minimization is restrained to the input protein coordinates by a user-selected RMSD tolerance.

Figure-9[191]

5(e) Crystal structure of Cyclopropane mycolic acid synthase – 2 enzymes: 1KPI

LIGAND PREPARATION [194,195]

The ligand preparation is designed to prepare a high quality, 3D structures for a large numbers of drugs like molecules. The structures which to be docked must have actual ligand structures and should meet the following conditions:

1. The structures must be in three dimensional.
2. The structures must have realistic bond lengths and bond angles.

3. Each structure must consist of a single molecule that has no covalent bonds to the receptor, with not accompanying fragments such as counter ions and solvent molecules.

4. All structures must have their hydrogens.

5. The structures must have an appropriate protonation state for the physiological pH values (around 7).

The LigPrep process consists of a series of steps which perform conversions, eliminate unwanted structures, apply corrections to the structures, and optimize the structures. The simple use of LigPrep produces a single low-energy 3D structure with correct chiralities for every successfully processed input structure. The LigPrep can also produce a large number of structures from each input structure with various ionization states, stereochemistries, and ring conformations, tautomers, and eliminate molecules using various criteria including molecular weight, specified numbers and types of functional groups which are present.

RECEPTOR GRID GENERATION [196]

The grid in which the shape and properties of the receptor are represented by a several different sets of fields which provide progressively more accurate scoring of the ligand poses. Grids must be prepared for each conformation to ensure that possible actives are not missed for receptors that adopt more than one conformation on binding.

Receptor grid defines the receptor structure by excluding any co-crystallised ligand which might be present, determine the position and the size of active site as it will be represented by receptor grids, set up the glide constraints, and set up the flexible hydroxyl groups.

The receptor grid generation requires a prepared structure where an all-atom structure with appropriate bond orders and formal charges.

LIGAND DOCKING [197]

Glide ligand docking job requires a set of previously calculated receptor grids and one or more ligand structures. The ligand structures must satisfy the criteria listed above in the ligand preparation. The detailed information on setting up grid generation job is given above. The preparation of ligands before docking is strongly recommended. The LigPrep or Macro model in Maestro can be used to prepare ligands. If a correct Lewis structure cannot be generated for a ligand, it is skipped by the docking job.

DOCKING OF CmaA-2[198,199,200]

Crystal structure of CmaA2 (PDB code-1KPI) was used for the study. The 3D structures was downloaded from the Protein Data Bank (PDB) and imported to the Maestro® workflow. Hydrogen atoms were added to the proteins and further the force field and other minimization was performed using protein preparation wizard. The structure based docking studies of CmaA2 inhibitors were carried out using Glide software to the 3D structure of CmaA2 and generated 10 best docking poses. During the docking studies, Glide initially performs a complete systematic search of conformations, orientations, and the position of a compound in the defined active binding site and eliminates the unwanted poses using scoring and energy optimization. The best poses were selected based upon the scoring functions and the quality of pose orientation within the active binding site amino acids. The molecules selected for the synthesis were examined from this docking score which is having a high dock value.

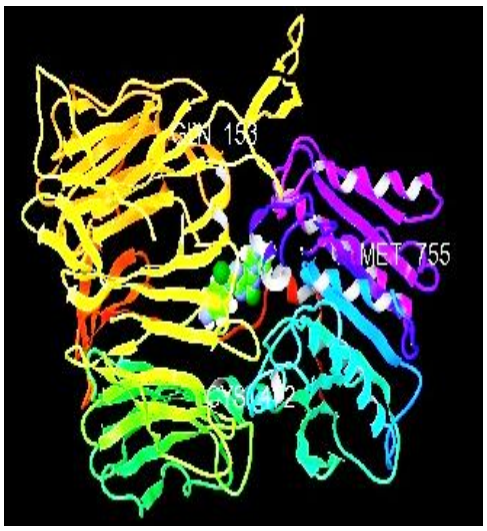


Figure-10[198]

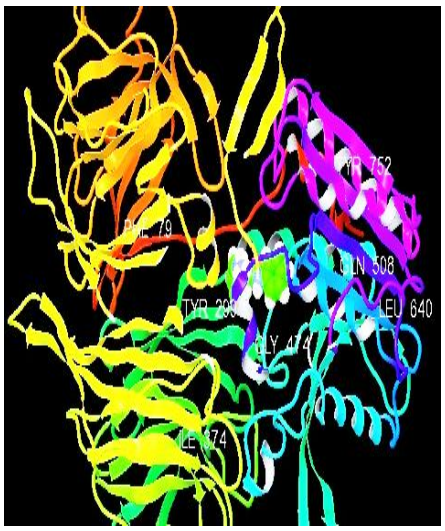


Figure-11[200]

Docked molecule in the cocrystal of CmaA2 receptor in ribbon form

COCRYSTAL AND DOCKING [201]

Co-crystal and docking were the conformational comparison of these two ligands having similar binding modes at the CmaA2 binding site.

Crystal structure of CmaA2 (Pdb code: 1KPI) was downloaded from the Protein data bank into the maestro workflow. The protein was then split by molecule and generates the co-crystal. The co-crystal alone was subjected to LigPrep. The output file was further docked and then generates docked conformations.

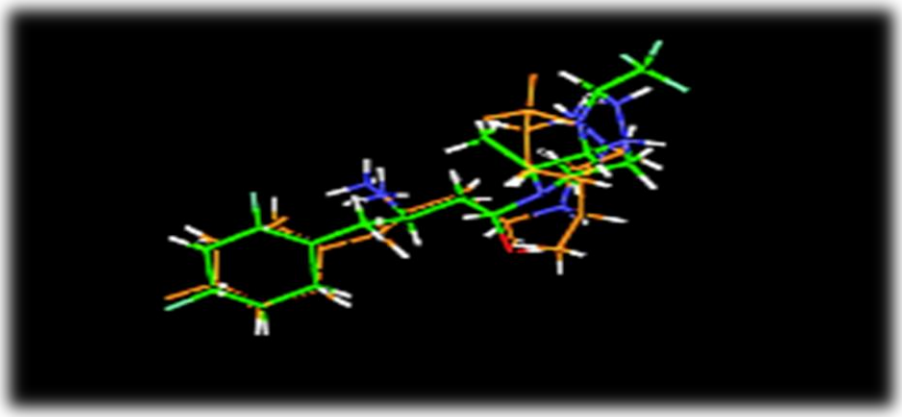
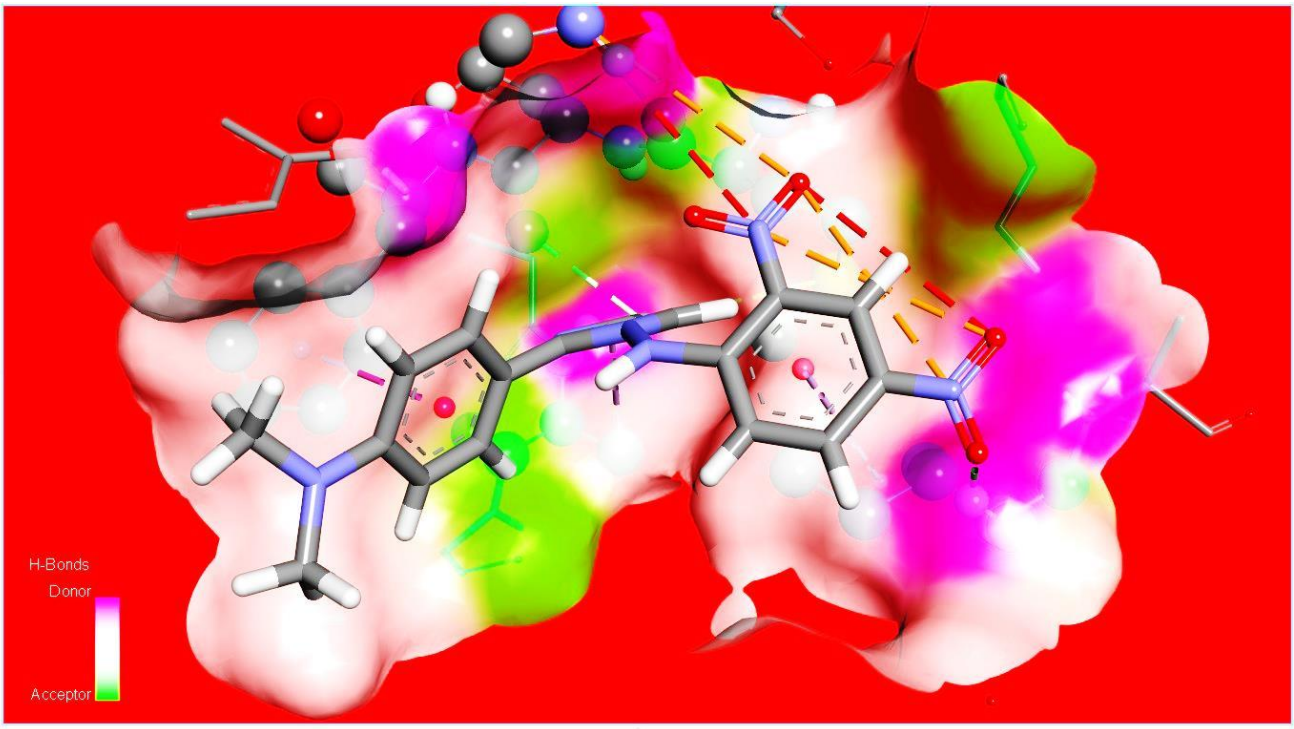


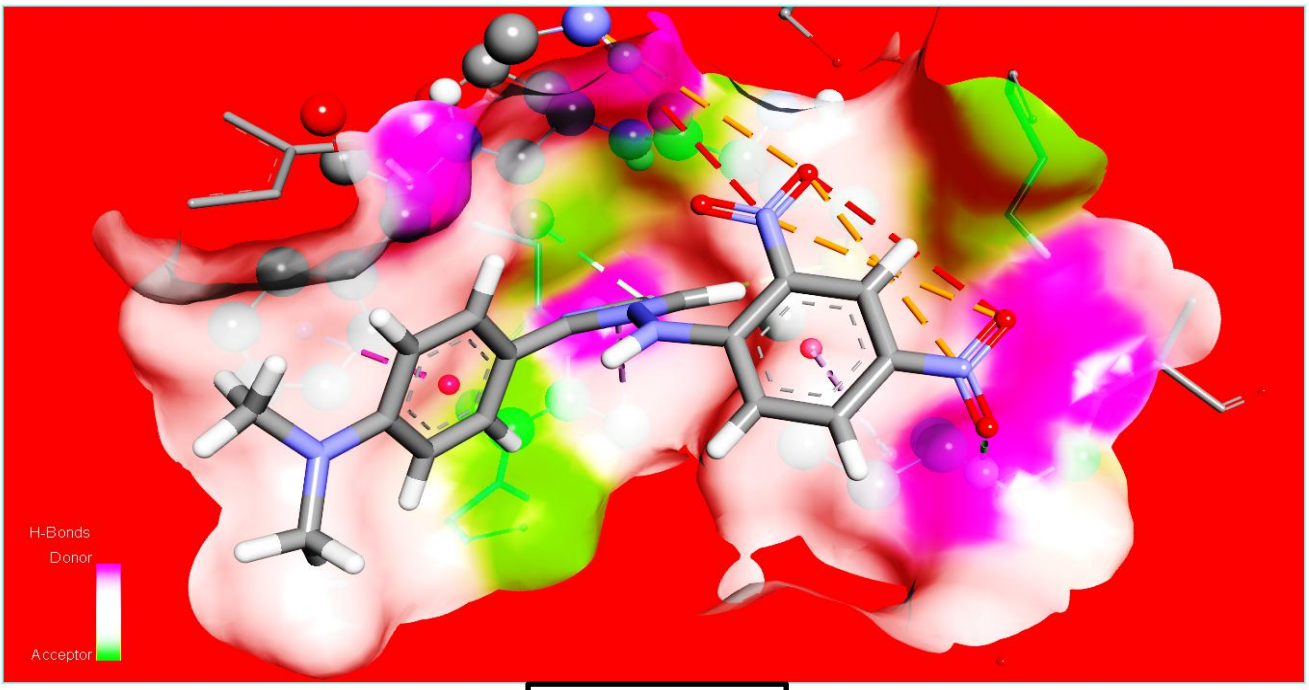
Figure-12 Co-crystal and docked orientation

INTERACTIONS

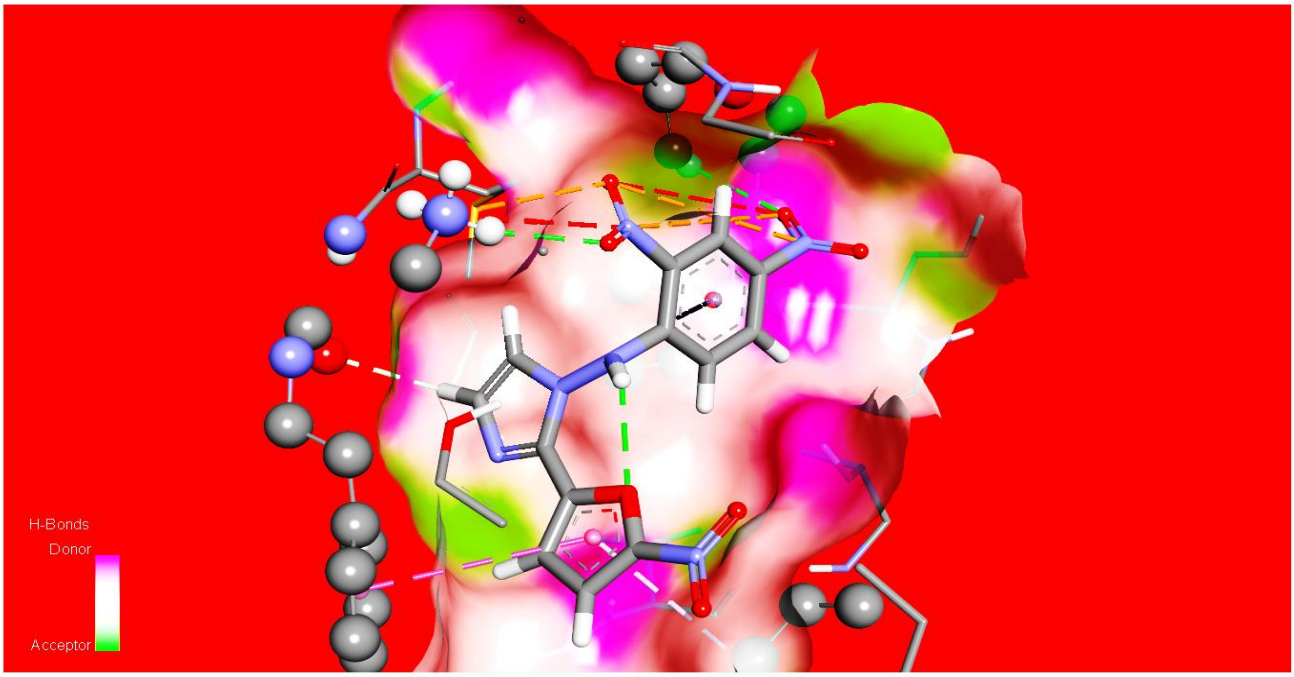
The binding modes of compounds SU03, SU 04, SU 05, SU 06,SU 07,SU 08,SU 09,SU 10,SU 11,and SU 12 in the active site of CmaA2. Carbon atoms of the protein and the ligand are indicated in gray. Each dotted line indicates a hydrogen bond.



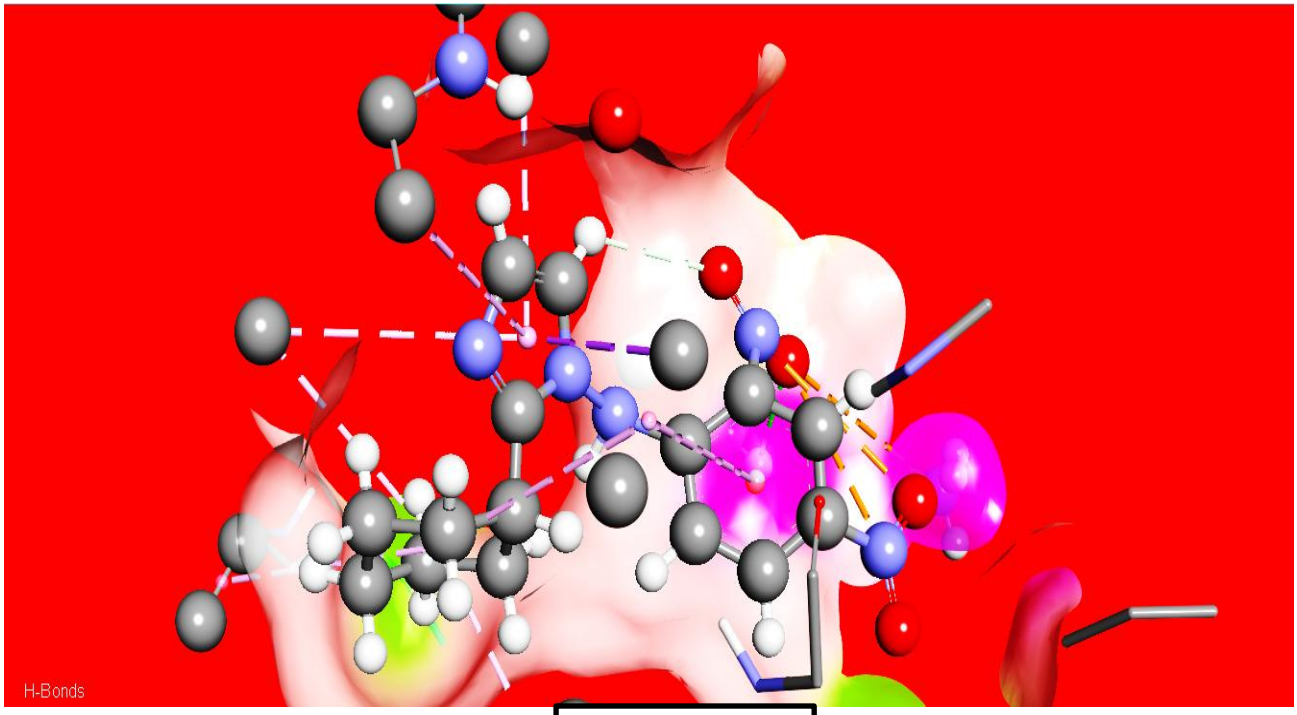
SU-1



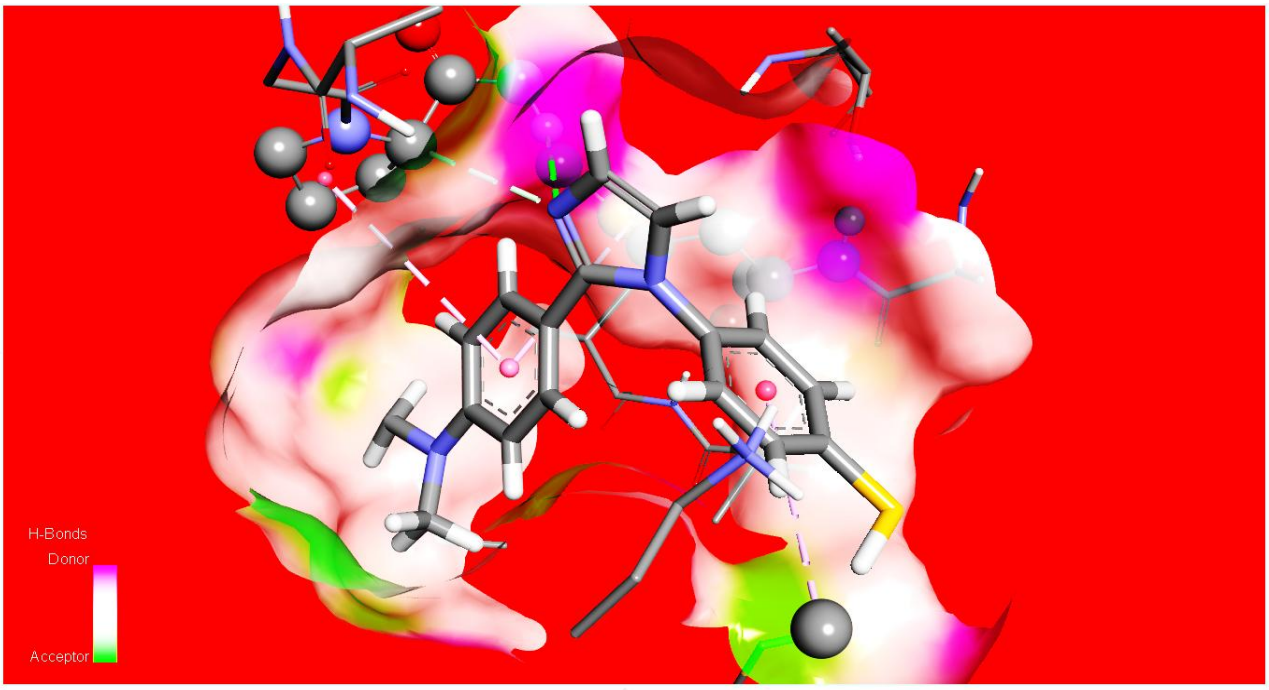
SU-2



SU-3

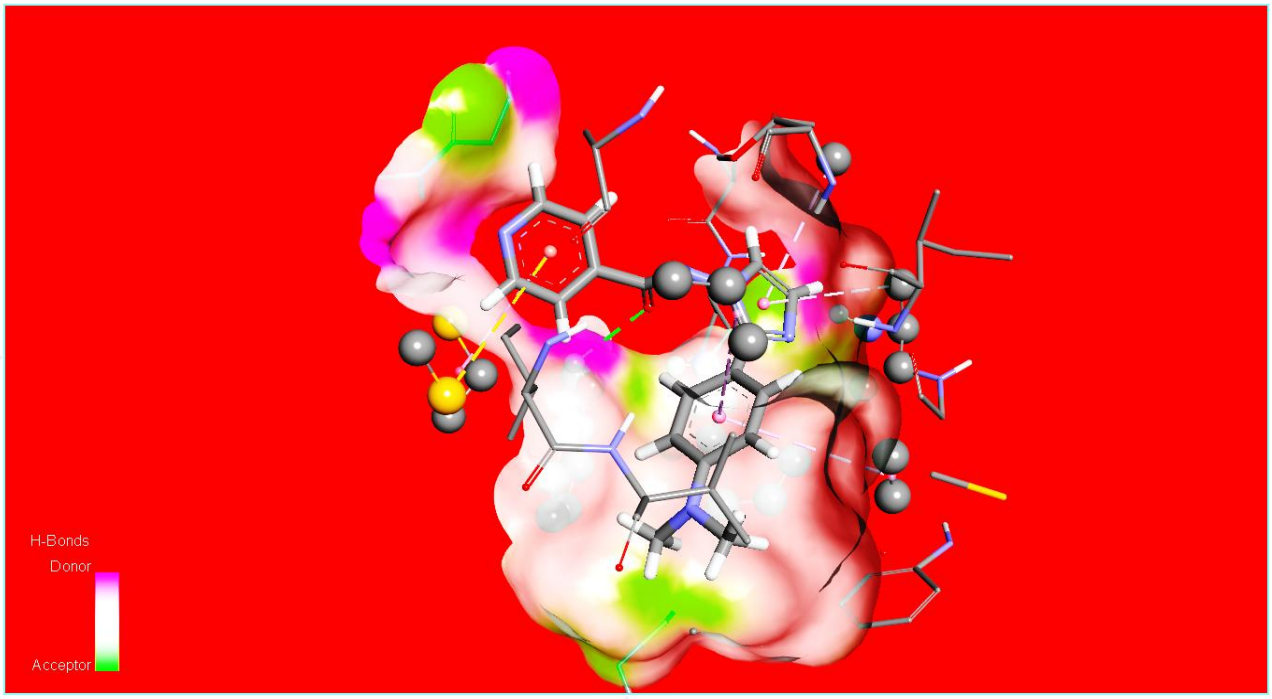


SU-4

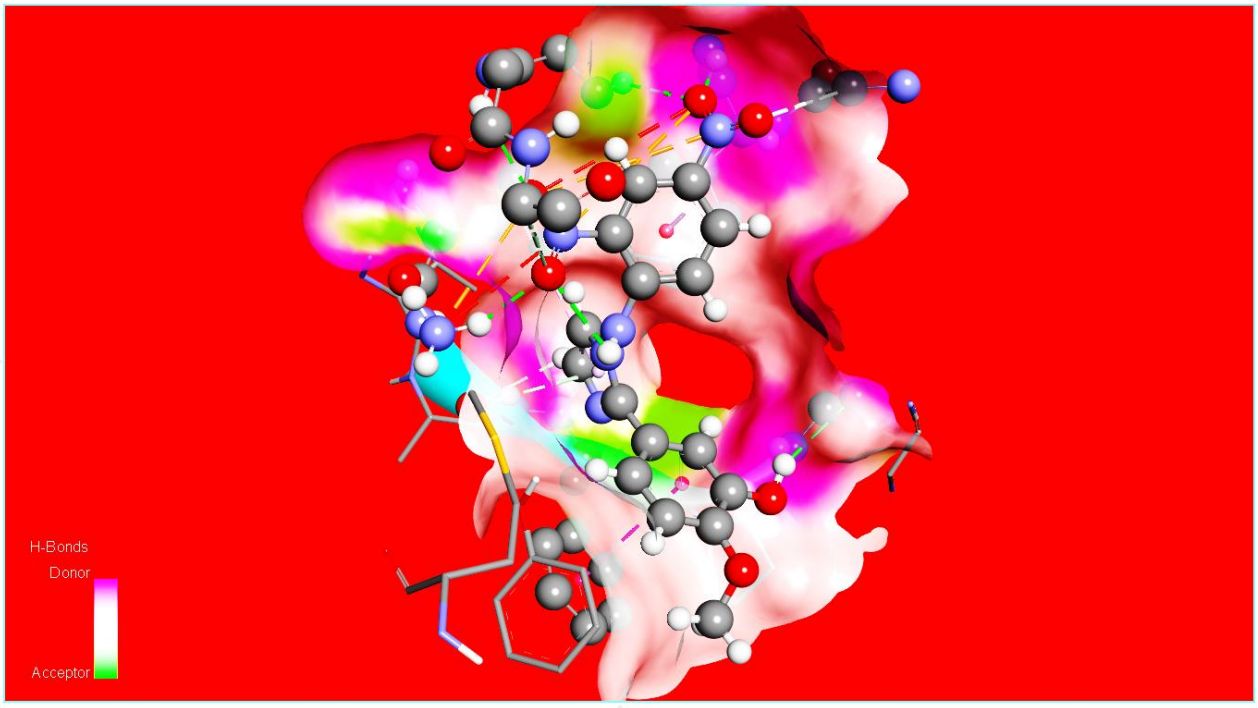


SU-5

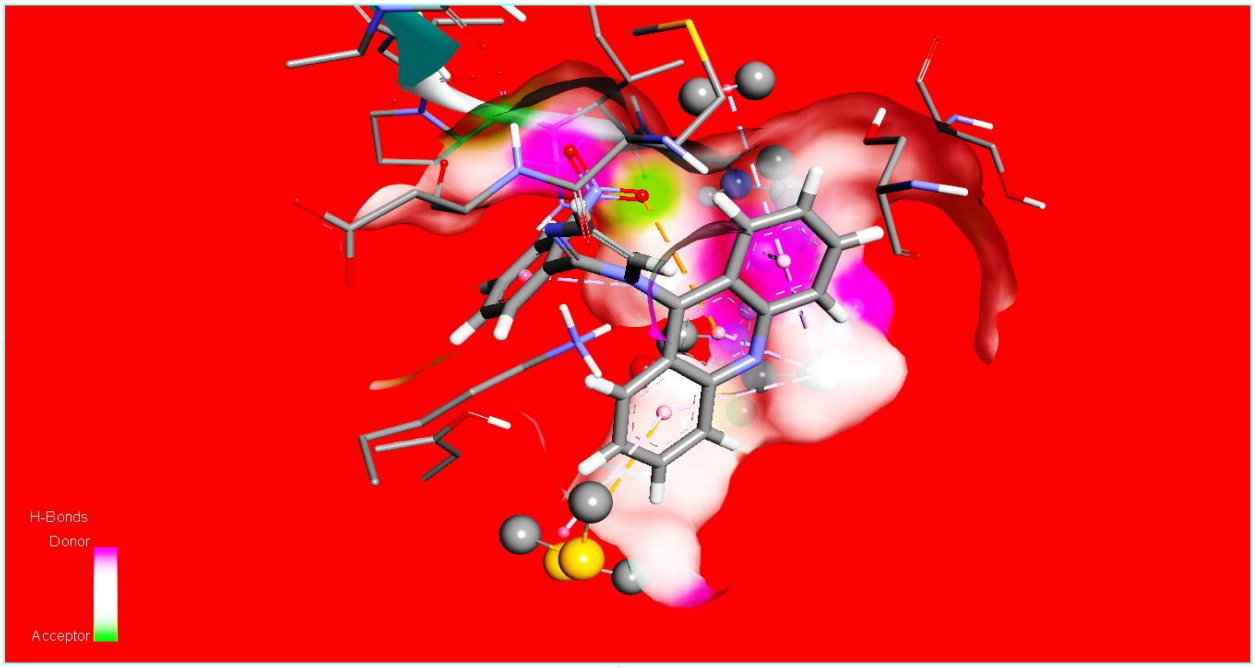
SU-6



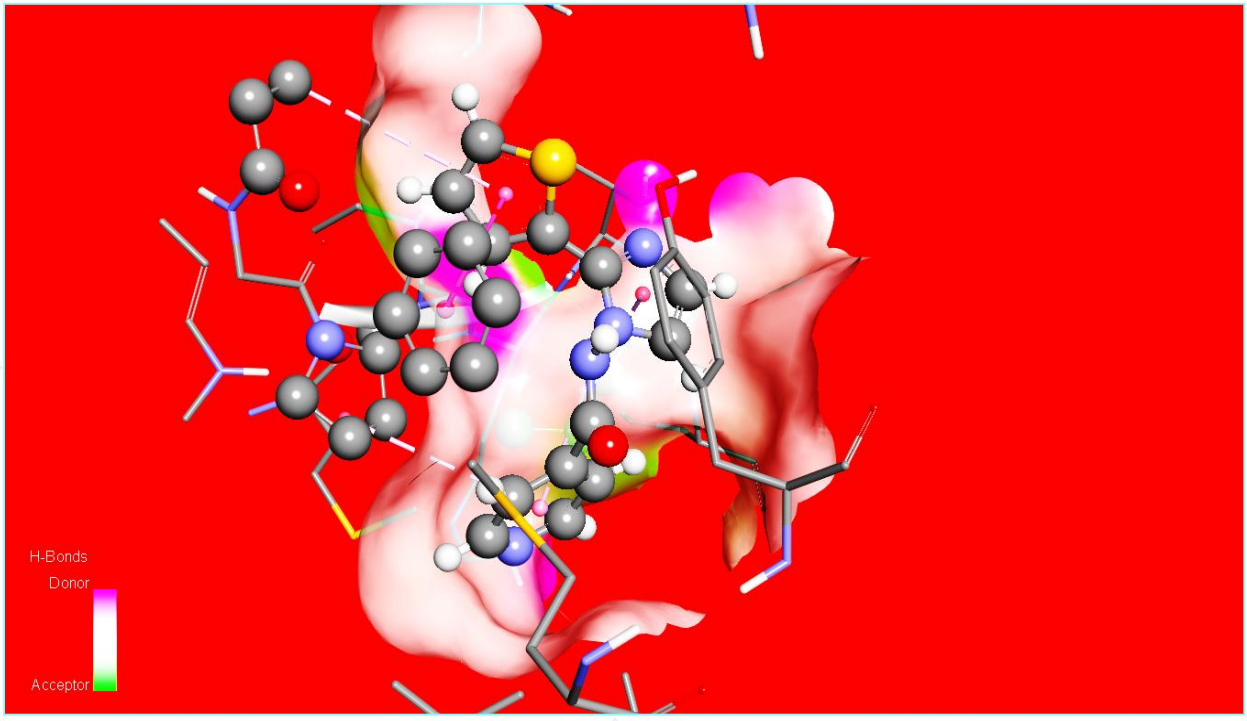
SU-7



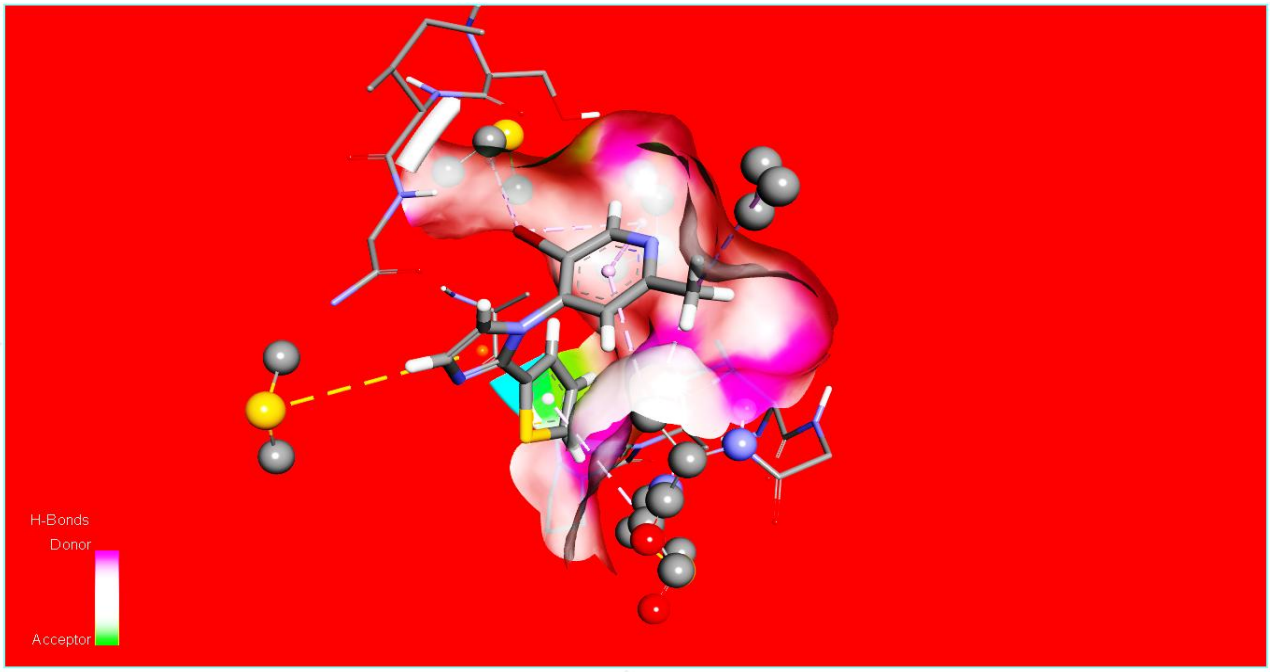
SU-8



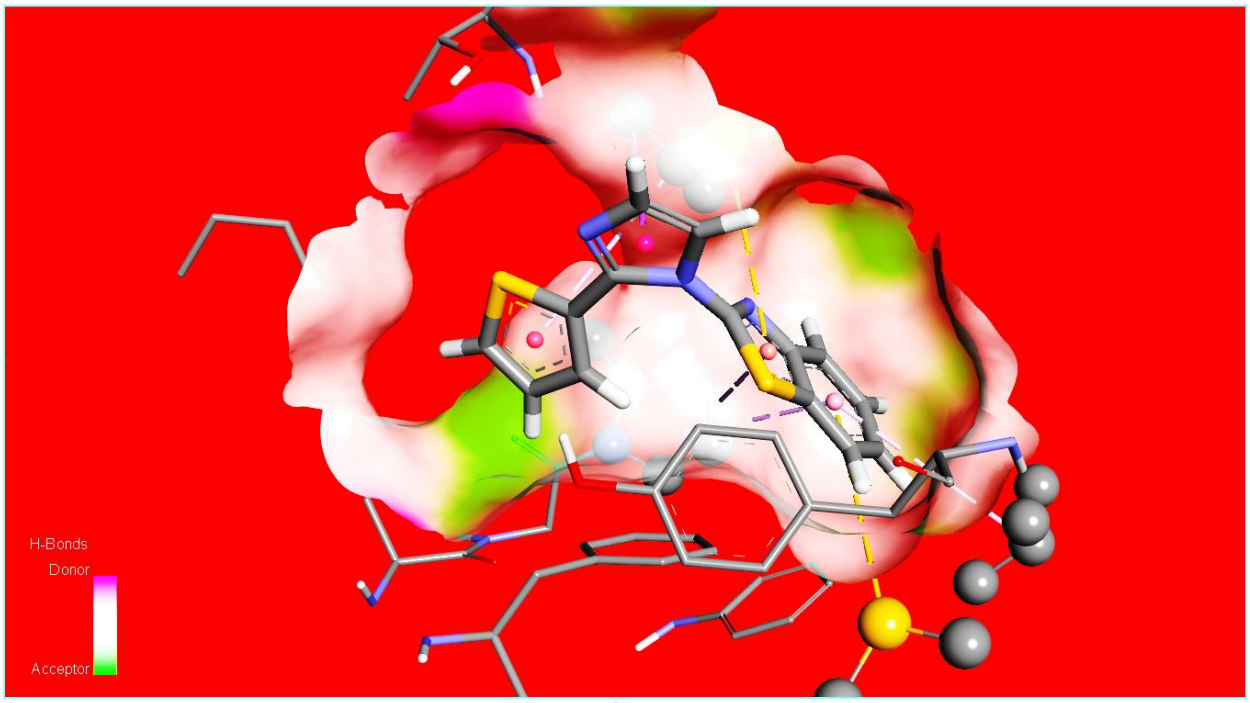
SU-9



SU-10



SU-11



SU-12

INTERACTION BETWEEN PROTEIN AND LIGAND

- Interaction between protein and ligand su-1
- Interaction between protein and ligand su-2
- Interaction between protein and ligand su-3
- Interaction between protein and ligand su-4
- Interaction between protein and ligand su-5
- Interaction between protein and ligand su-6
- Interaction between protein and ligand su-7
- Interaction between protein and ligand su-8
- Interaction between protein and ligand su-9
- Interaction between protein and ligand su-10
- Interaction between protein and ligand su-11
- Interaction between protein and ligand su-12

XP DOCKING OF MOLECULES GOING TO BE SYNTHESIZED [202]

Docking of selected molecules from the scaffhold is done by XP mode against 1kpi for evaluating the degree of binding by checking or evaluates the following parameters for detailed study with the receptor.

Table-3

S.NO	XP Term	Description
1	GScore	Total GlideScore; sum of XP Terms
2	LipophilicEvdW	Lipophilic term derived from hydrophobic grid potential and fraction of the total protein ligandvdW energy
3	PhobEn	Hydrophobic enclosure reward
4	PhobEnHB	Reward for hydrophobically packed H-bond
5	PhobEnPairHB	Reward for hydrophobically packed correlated H-bonds
6	HBond	ChemScore H-bond pair term
7	Electro	Electrostatic rewards
8	SiteMap	SiteMap ligand/receptor non-Hbonding polar/hydrophobic and hydrophobic/hydrophilic complementarity Terms
9	LowMW	Reward for ligands with low molecular weight
10	Penalties	Polar atom burial and desolvation penalties, and penalty for intra-ligand contacts
11	HBPenal	Penalty for ligands with large hydrophobic contacts and low H-bond scores
12	PhobicPenal	Penalty for exposed hydrophobic ligand groups
13	RotPenal	Rotatable bond penalty

DOCKING SCORE-Table-4

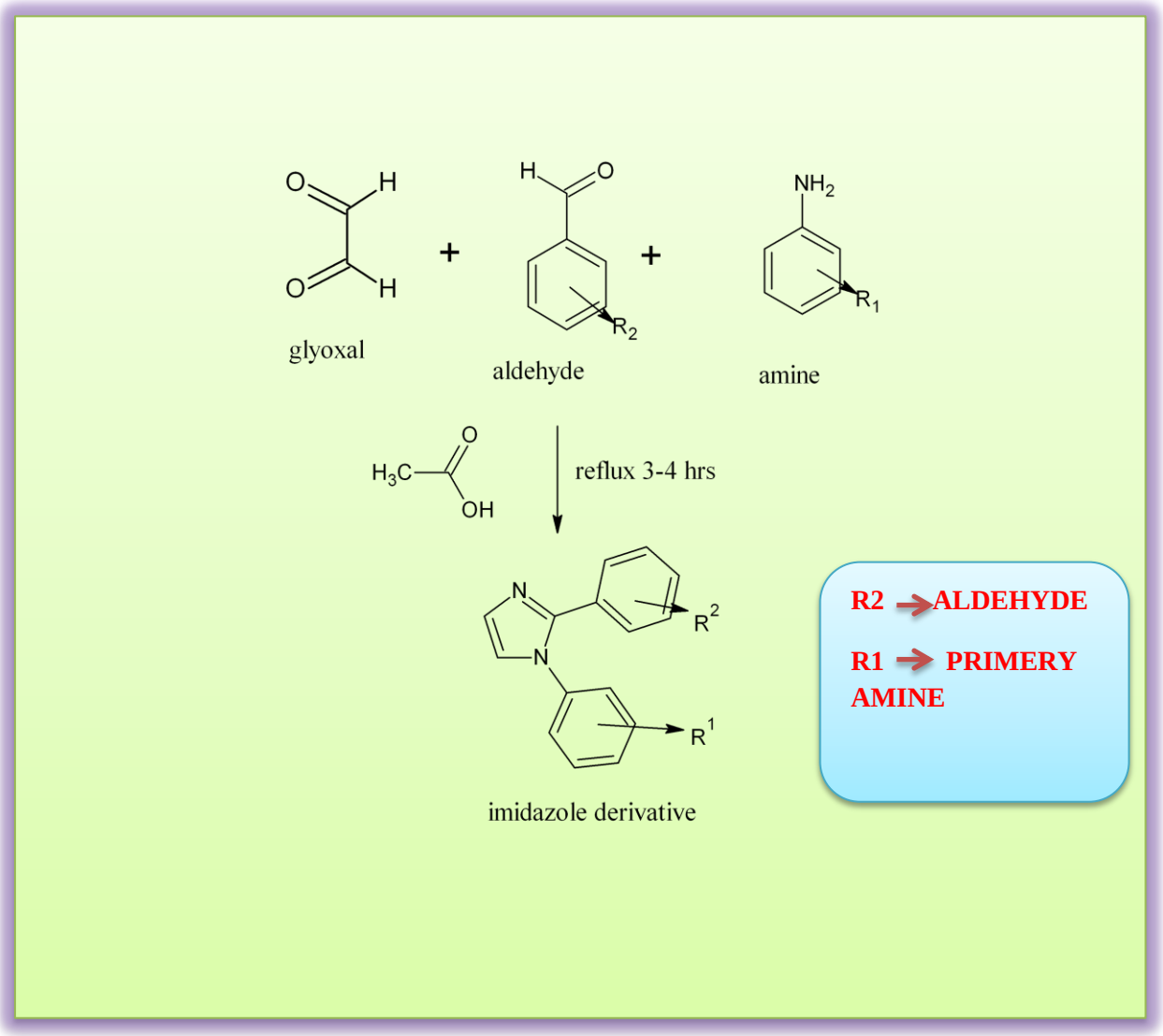
Ligand	G Score	Lipophilic Evdw	PhobEn	PhobEn HB	PhobEn PairHB	H bond	Elect	Site map	Pi Cat	ClBr	Low Mw
1	-8.0	-3.71	-1.22	-1.5	0	-1.36	-0.82	-0.03	0	0	-0.19
2	-10.0	-3.97	-1.55	0	0	-0.9	-0.11	-0.4	0	0	-0.38
3	-9.1	-4.08	-1.7	0	0	-0.35	-0.13	-0.26	0	0	-0.13
4	-6.04	-4.67	-1.20	0	0	0	-0.23	-0.31	0	0	-0.19
5	-6.8	-3.75	-1.57	0	0	0	-0.06	-0.21	0	0	-0.33
6	-5.9	-4.01	-1.2	0	0	-0.7	-1.84	-0.3	0	0	-0.5
7	-6.3	-4.68	-1.35	0	0	-0.7	-0.09	-0.3	0	0	-0.34
8	-7.4	-4.20	-1.3	0	0	-0.45	-0.06	-0.1	0	0	-0.5
9	-8.2	-3.92	-1.2	0	0	-0.7	-0.43	-0.5	0	0	-0.52
10	-7.6	-4.01	-1.25	0	0	0	-1.5	-0.9	0	0	-0.24
11	-8.6	-3.52	-1.5	0	0	-0.5	-1.2	-0.68	0	0	-0.51
12	-9.5	-3.68	-1.7	0	0	-0.1	-1.8	-0.23	0	0	-0.52

Synthetic investigation

It is a simple and expedient three component reaction with some modifications in dr. A.jread suresh involving glyoxal, substituted aldehyde and substituted amine in the presence ammonium acetate for the synthesis of substituted imidazoles.

Initially, it has been investigated a three component reaction of glyoxal**,** benzaldehyde and aniline in different solvent systems like methanol, ethanol, toluene and acetic acid and in presence of various bases like NH_3, triethylamine, ammonium acetate, pyridine under reflux in 70^0C to afford substituted imidazoles. The high yield of the product was obtained by the reaction mixture in acetic acid in presence of ammonium acetate. [203]

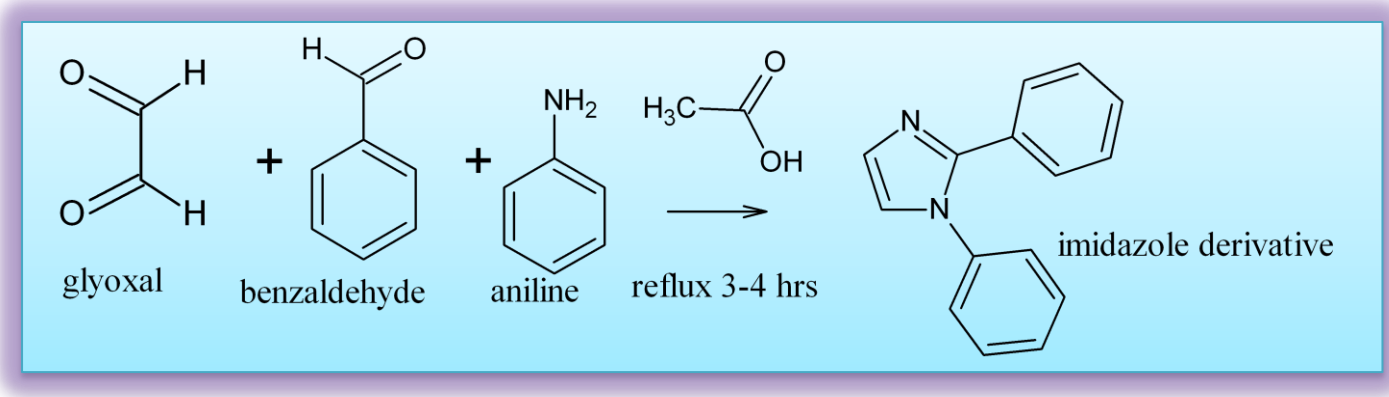
Synthetic scheme:



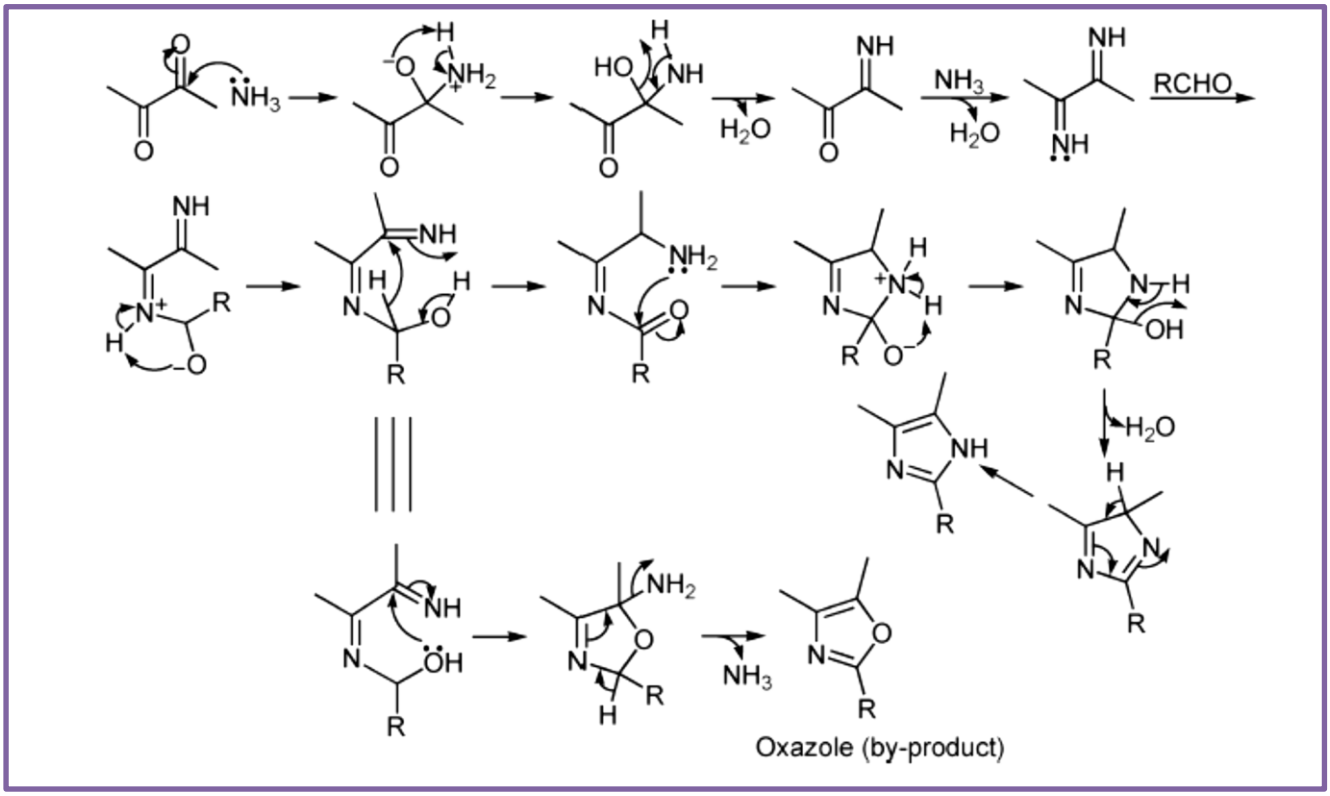
SYNTHETIC METHODS:

Synthesis of Compound: [204,205]

A mixture of glyoxal (1.1 mmol), benzaldehyde (1.35 mmol) and aniline (1.75mmol) in acetic acid was reflux. After the complete disappearance of the both starting materials (monitored by TLC), ammonium acetate was added and the feflux was continued for 2½-3 hrs. The completion of the reaction is then confirmed by the TLC. Then its transfer to cold ice and getting precipitated the solid is then separated by vacuum filtration and washed with ethanol. The compound is recrystallized using ethanol.

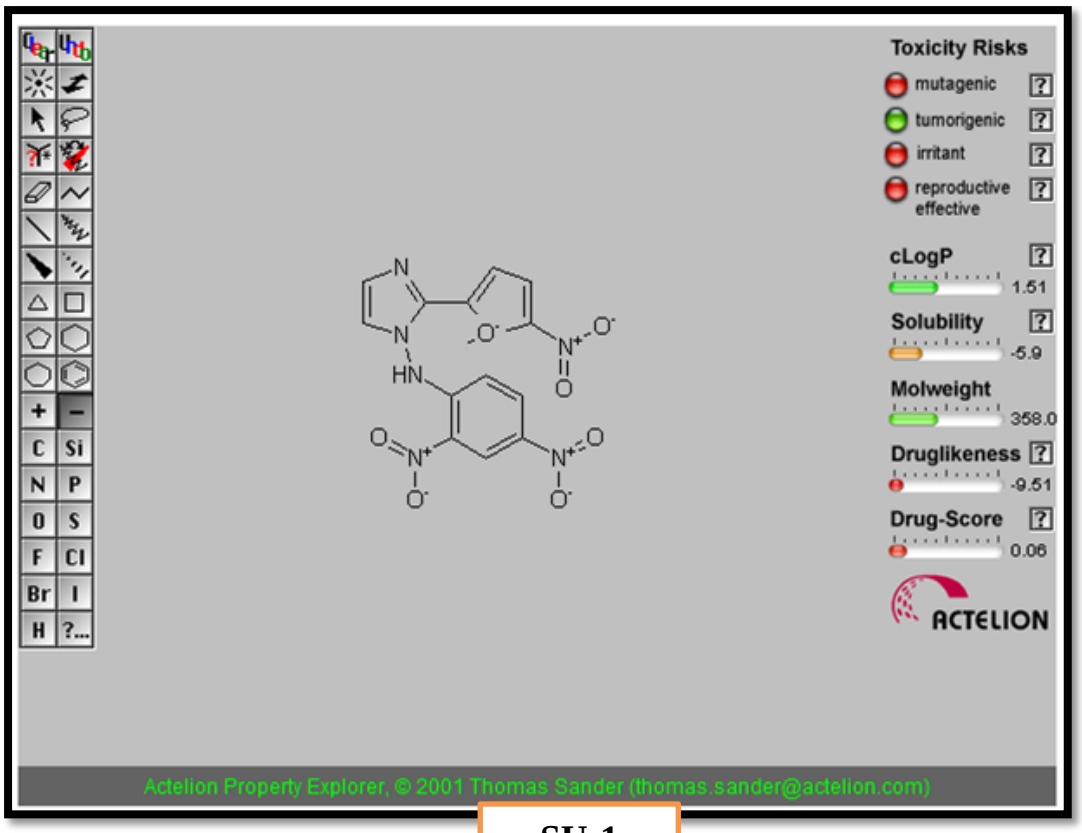


Mechanism for Formation of imidazole derivatives: [206]

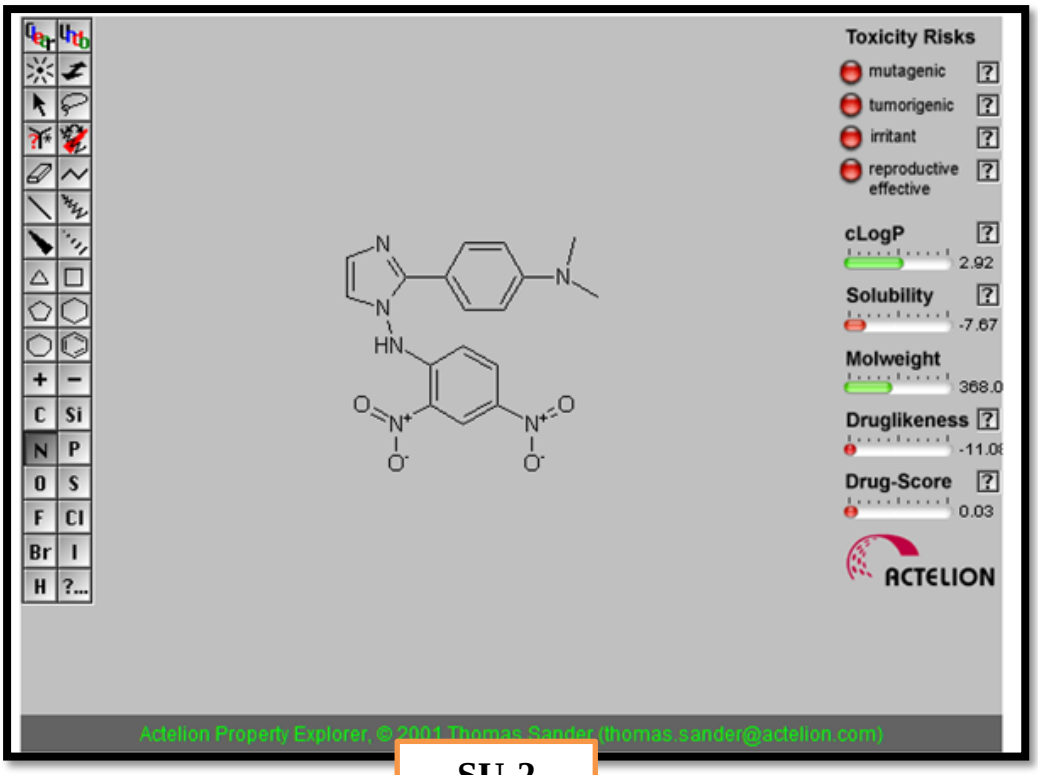


TOXICITY PREDICTION [207,208]

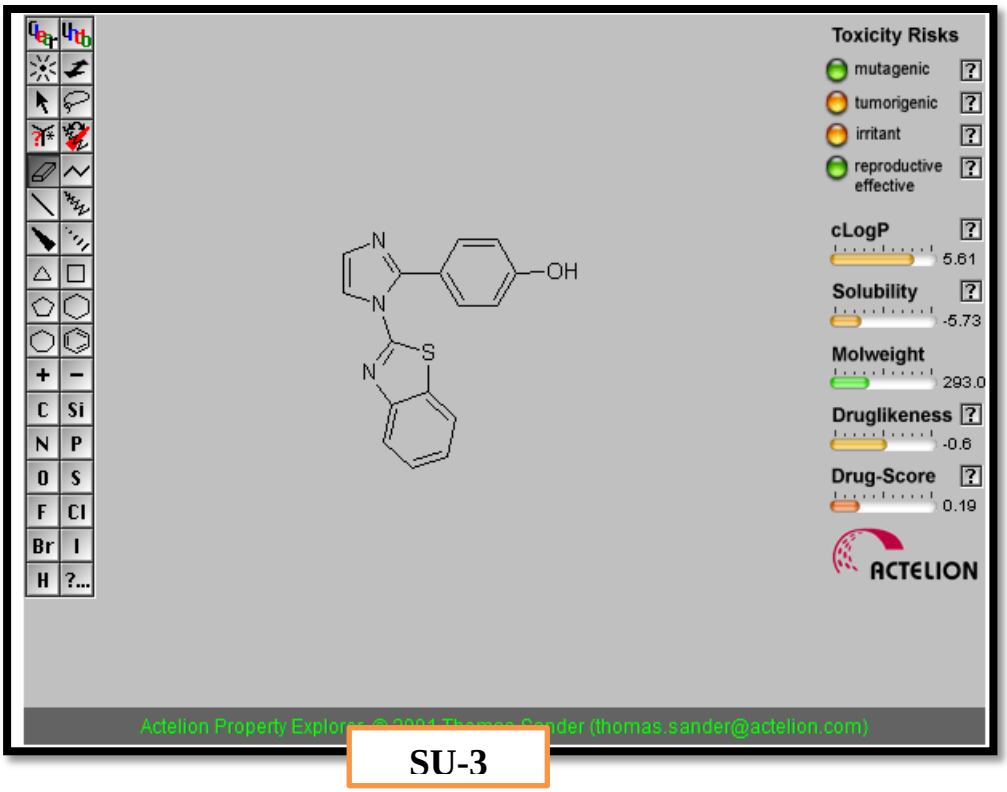
Toxicity predicted by the OSIRIS Property Explorer the online software of Thomas Sander, Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, and 4123 Allschwil, Switzerland. The OSIRIS Property Explorer shown in this page is an integral part of Actelion's (1) inhouse substance registration system. It lets you draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid. Prediction results are valued and color coded. Properties with high risks of **undesired effects** like mutagenicity or a poor intestinal absorption are shown in **red.** Whereas a **green** color indicates **drug-conform** behaviour.



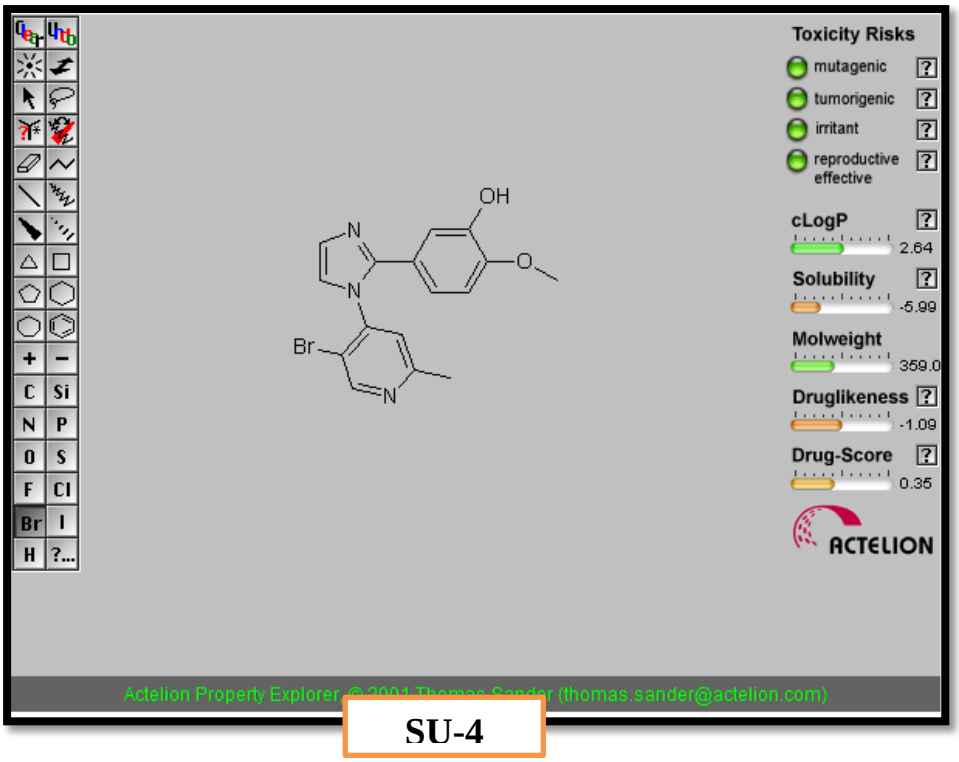
SU-1


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
2.92
Solubility
-7.67
Molweight
368.0
Druglikeness
Drug-Score
0.03
ACTELION
Actelion Property Explorer, © 2001 Thomas Sander (thomas.sander@actelion.com)

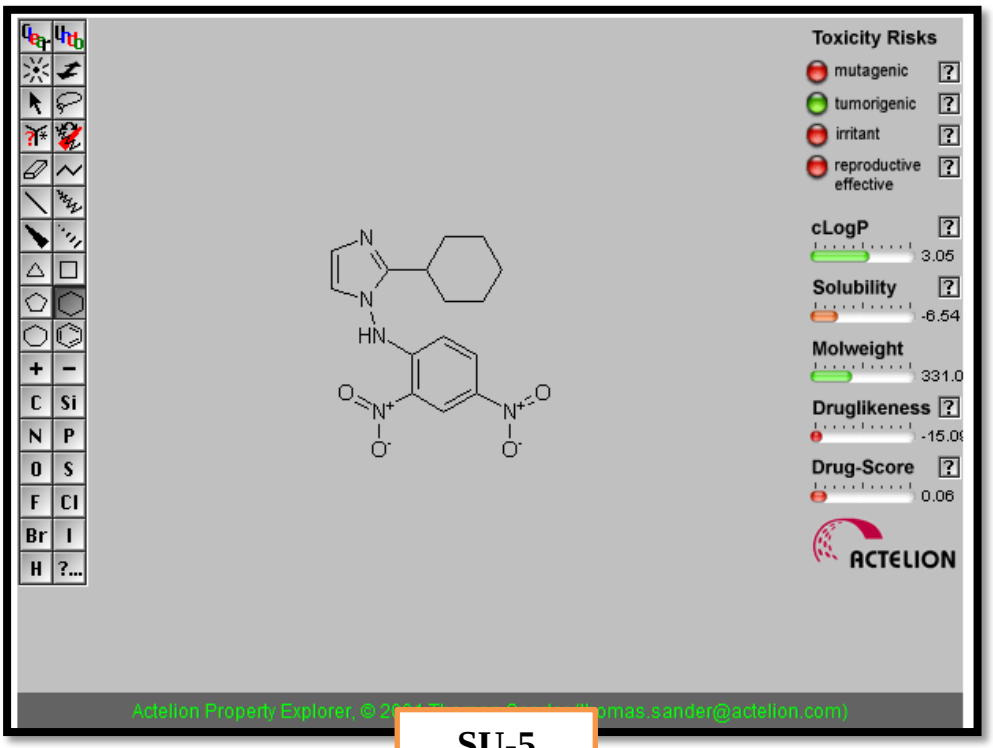
SU-2


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
5.61
Solubility
-5.73
Molweight
293.0
Druglikeness
-0.6
Drug-Score
0.19
ACTELION
OH
Actelion Property Explorer, © 2001 Thomas Sander (thomas.sander@actelion.com)

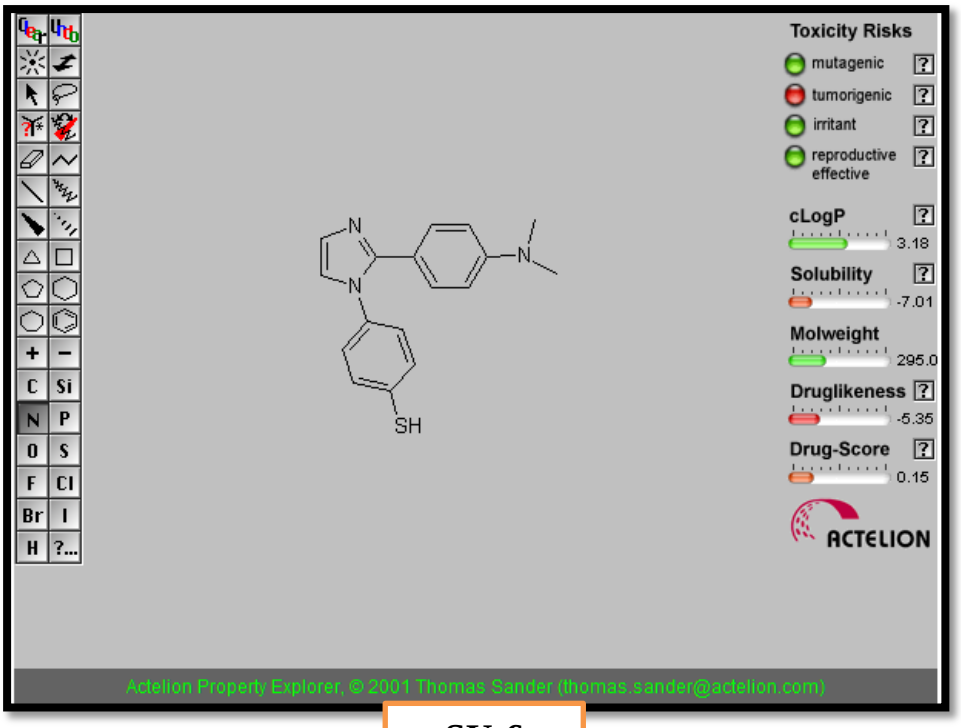
SU-3


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
2.64
Solubility
-5.99
Molweight
359.0
Druglikeness
-1.09
Drug-Score
0.35
ACTELION
OH
N
N
O
Br
N
Actelion Property Explorer

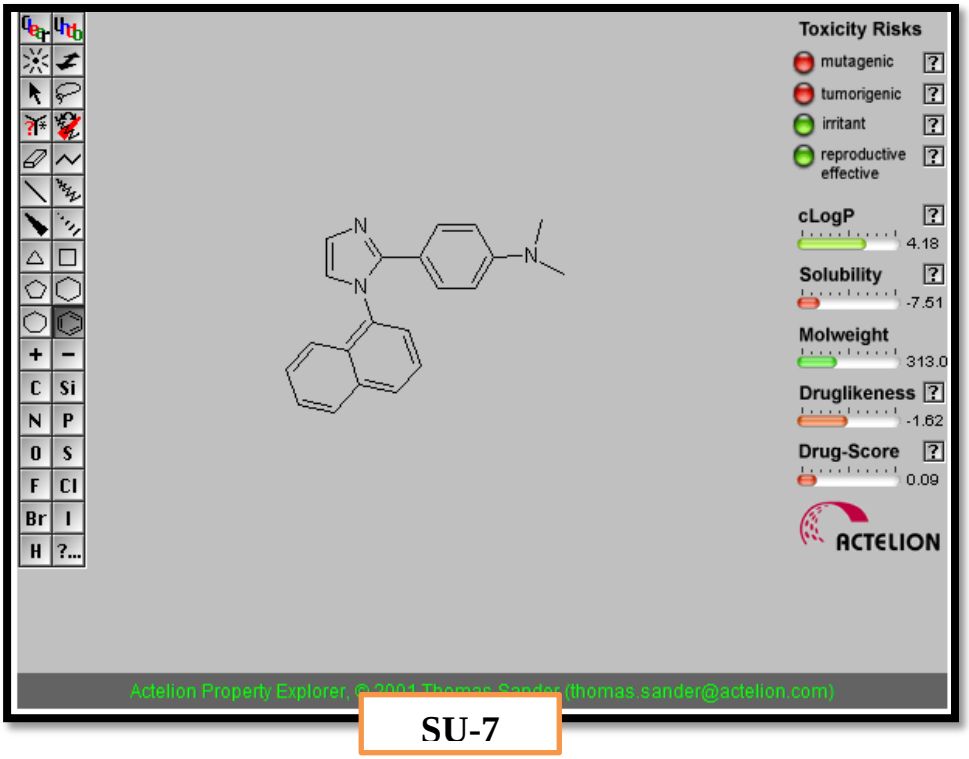
SU-4


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
3.05
Solubility
-6.54
Molweight
331.0
Druglikeness
Drug-Score
0.06
ACTELION
N
N
HN
O
N+
O-
N+
O
O-
Actelion Property Explorer

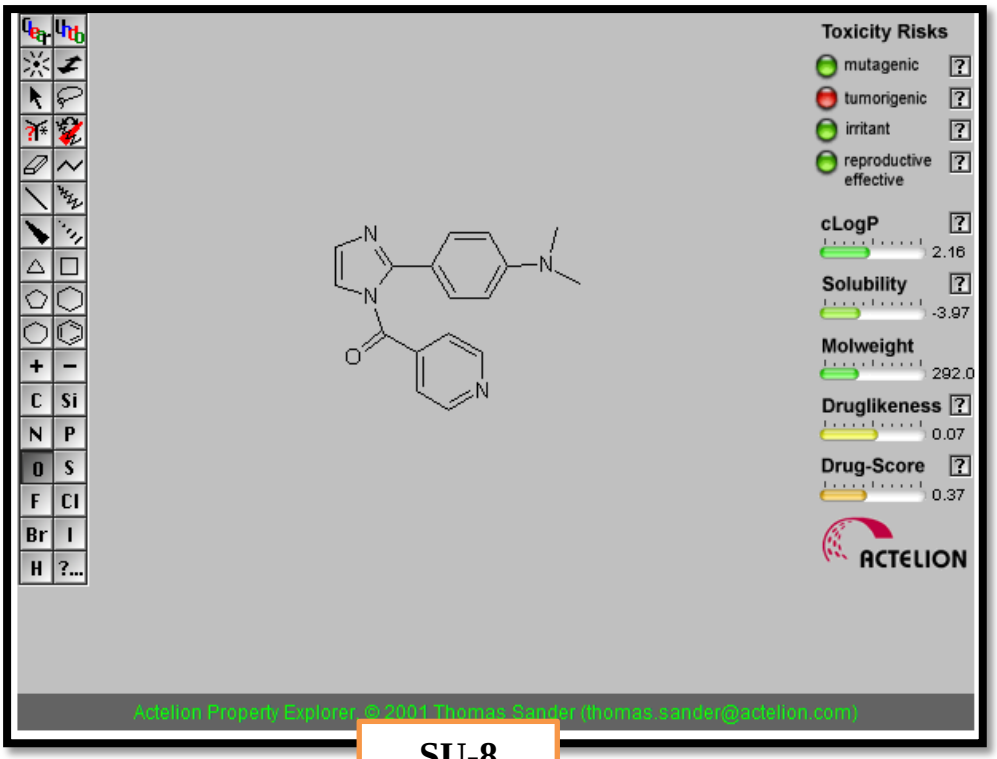
SU-5


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
3.18
Solubility
-7.01
Molweight
295.0
Druglikeness
-5.35
Drug-Score
0.15
ACTELION
SH
Actelion Property Explorer, © 2001 Thomas Sander (thomas.sander@actelion.com)

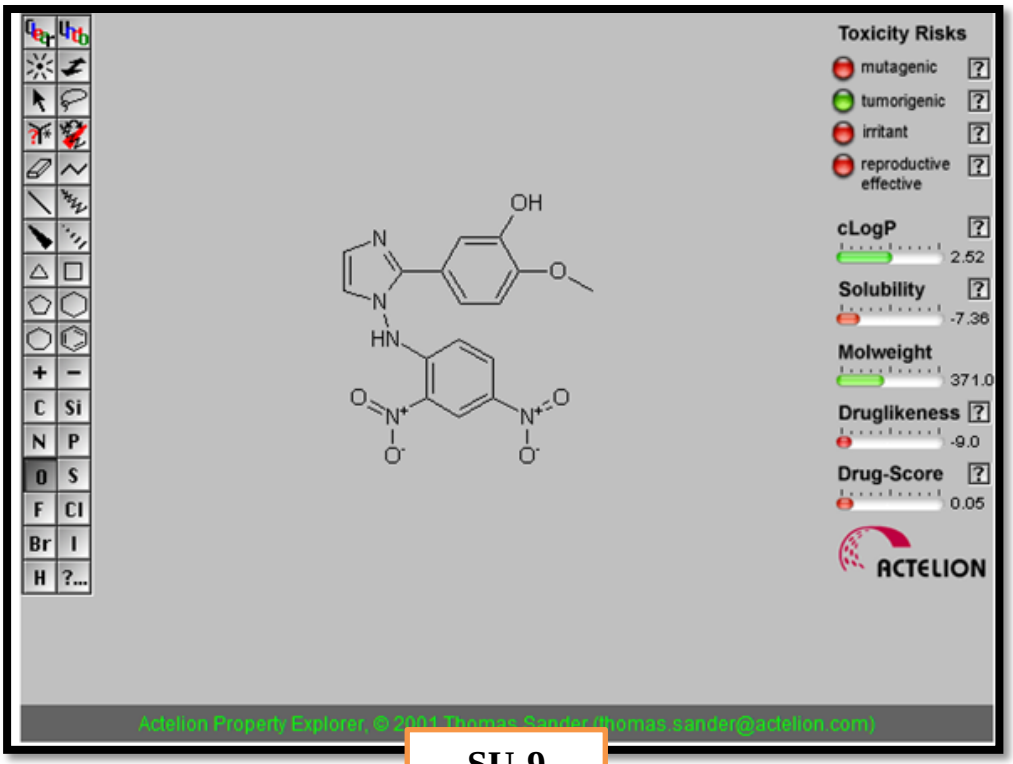
SU-6


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
4.18
Solubility
-7.51
Molweight
313.0
Druglikeness
-1.62
Drug-Score
0.09
ACTELION
Actelion Property Explorer, © 2001 Thomas Sander (thomas.sander@actelion.com)

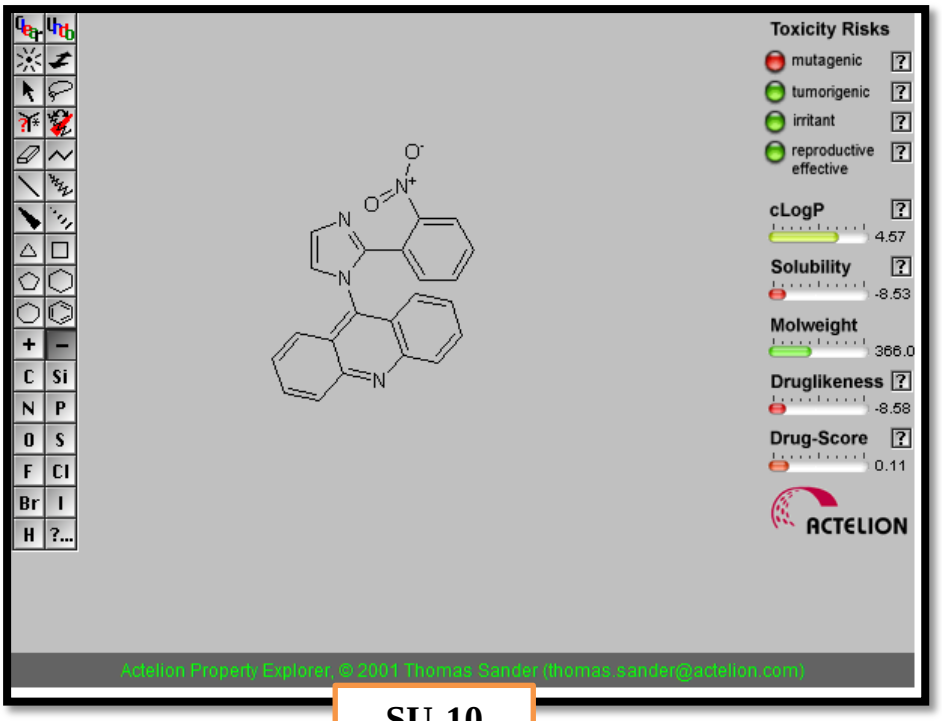
SU-7


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
2.16
Solubility
-3.97
Molweight
292.0
Druglikeness
0.07
Drug-Score
0.37
ACTELION
Actelion Property Explorer, © 2001 Thomas Sander (thomas.sander@actelion.com)

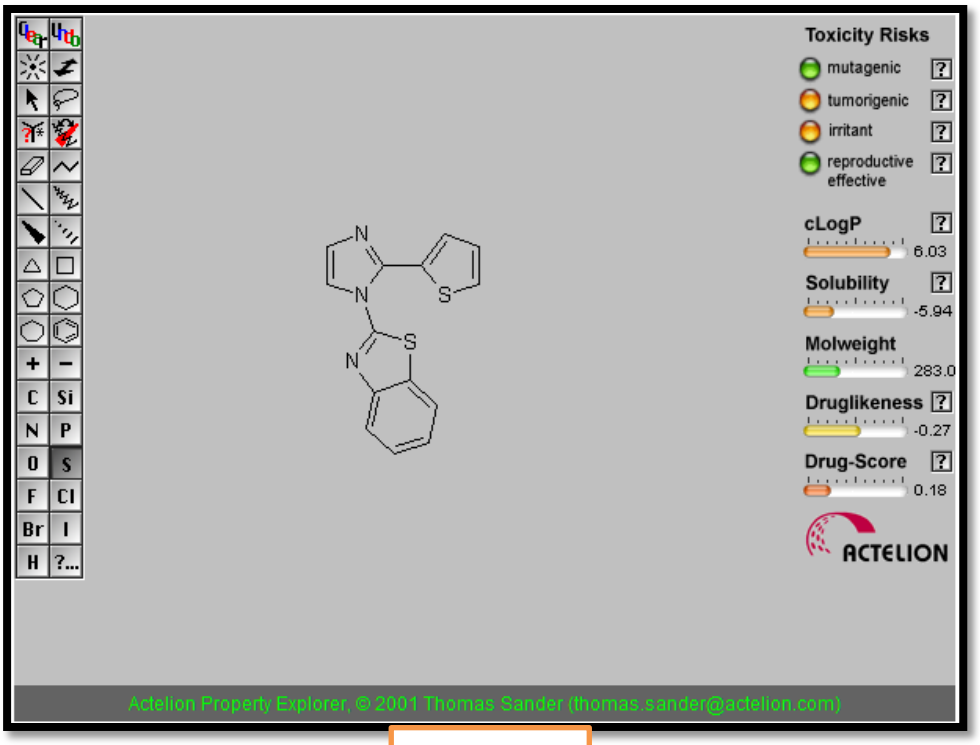
SU-8


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
2.52
Solubility
-7.36
Molweight
371.0
Druglikeness
-9.0
Drug-Score
0.05
ACTELION
Actelion Property Explorer, © 2001 Thomas Sander (thomas.sander@actelion.com)

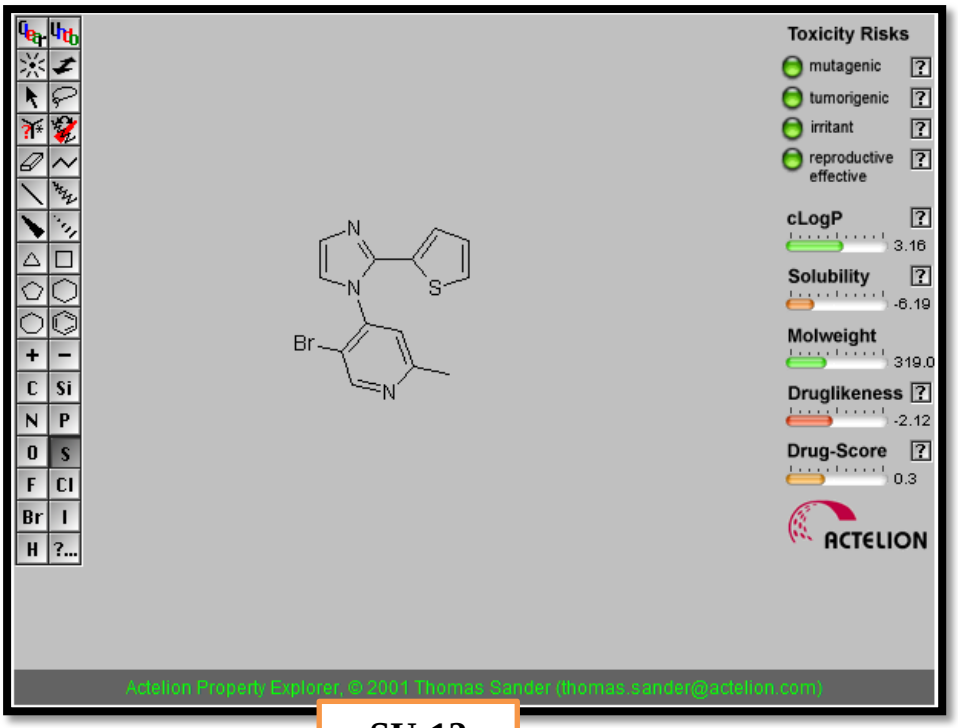
SU-9


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
4.57
Solubility
-8.53
Molweight
366.0
Druglikeness
-8.58
Drug-Score
0.11
ACTELION
Actelion Property Explorer, © 2001 Thomas Sander (thomas.sander@actelion.com)

SU-10


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
6.03
Solubility
-5.94
Molweight
283.0
Druglikeness
-0.27
Drug-Score
0.18
ACTELION
Actelion Property Explorer, © 2001 Thomas Sander (thomas.sander@actelion.com)

SU-11


Toxicity Risks
mutagenic
tumorigenic
irritant
reproductive effective
cLogP
3.16
Solubility
-6.19
Molweight
319.0
Druglikeness
-2.12
Drug-Score
0.3
ACTELION
Actelion Property Explorer, © 2001 Thomas Sander (thomas.sander@actelion.com)
N
N
S
Br
N

SU-12

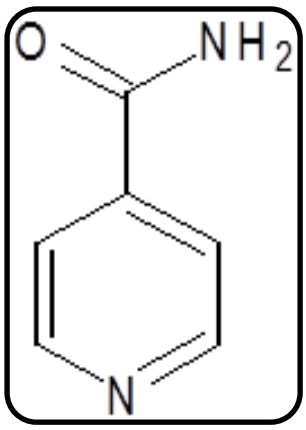
INVITRO ANTI TUBERCLAR ACTIVITY [209,210]

Anti-TB activity using Alamar Blue Dye

- The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).
- This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.
- Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
- The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
- The final drug concentrations tested were 100 to 0.2 µg/ml.
- Plates were covered and sealed with parafilm and incubated at 37ºC for five days.
- After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.
- A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
- The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

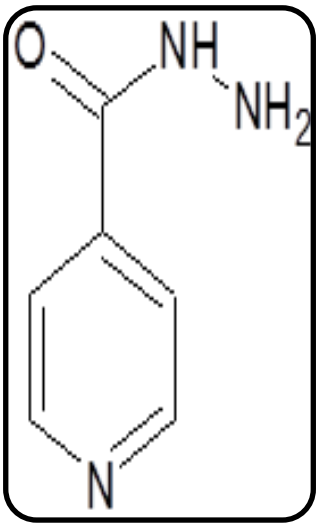
Reactant profile

ISONICOTINAMIDE



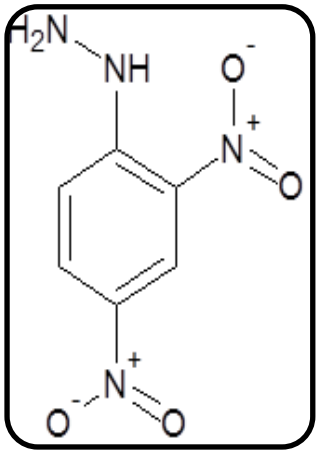
IUPAC NAME	:	Pyridine-4-carboxamide
Molecular Formula	:	$C_6H_6N_2O$
Formula Weight	:	122.12
Description	:	White crystalline powder
Melting point	:	128 C
Solubility	:	ethanol

ISONIAZID



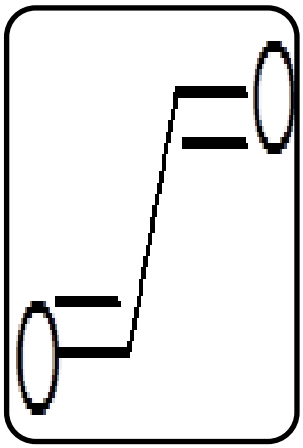
IUPAC NAME	:	Pyridine-4-carboxamide
Molecular Formula	:	$C_6H_7N_3O$
Formula Weight	:	137.13
Description	:	White crystalline powder
Melting point	:	197-200 C
Solubility	:	ethanol

2, 4-DINITROPHENYLHYDRAZINE



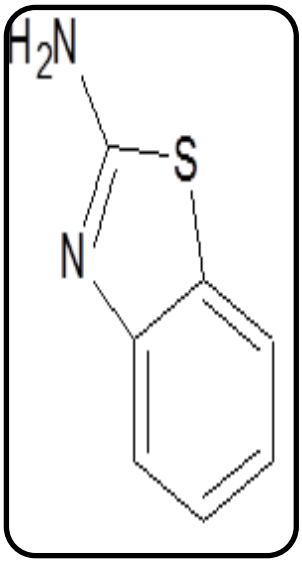
IUPAC NAME : (2, 4-dinitrophenyl) hydrazine
Molecular Formula : $C_6H_6N_4O_4$
Formula Weight : 198.13
Description : yellow crystalline powder
Melting point : 197-200 C
Solubility : ethanol

GLYOXAL



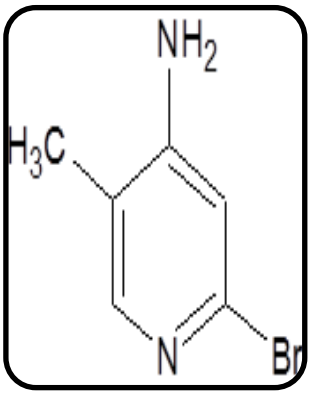
IUPAC NAME : ethane dials
Molecular Formula : $C_2H_2O_2$
Formula Weight : 58.04
Description : 40% aqueous solution
Melting point : 15 C
Solubility : ethanol

2-AMINOBENZOTHIAZOLE



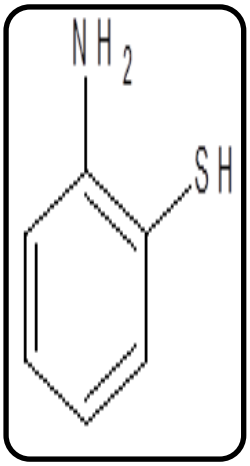
IUPAC NAME	:	1, 3-benzothiazol-2-amine
Molecular Formula	:	$C_7H_6N_2S$
Formula Weight	:	150.20
Description	:	yellow color powder
Melting point	:	129 C
Solubility	:	ethanol

2-BROMO-5-METHYLPYRIDINE



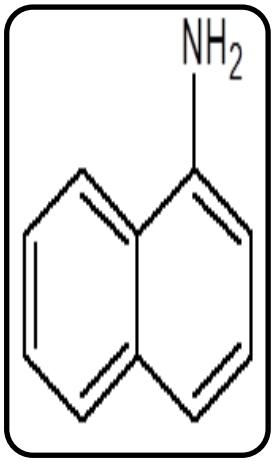
IUPAC NAME	:	2-bromo-5-methylpyridin-4-amine
Molecular Formula	:	C_6H_6BrN
Formula Weight	:	172.20
Description	:	light yellow color powder
Melting point	:	43 C
Solubility	:	ethanol

2- AMINOTHIOPHENOL



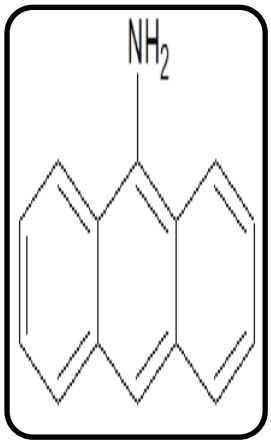
IUPAC NAME : 2-bromo-5-methylpyridin-4-amine
Molecular Formula : C_6H_7NS
Formula Weight : 125.20
Description : color less liquid
Melting point : 23 C
Solubility : ethanol

1- NAPHTHYLAMINE



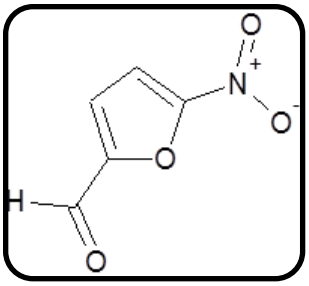
IUPAC NAME : naphthalen-1-amine
Molecular Formula : $C_{10}H_9N$
Formula Weight : 143.20
Description : light purple
Melting point : 50 C
Solubility : ethanol

9- AMINOACRIDINE



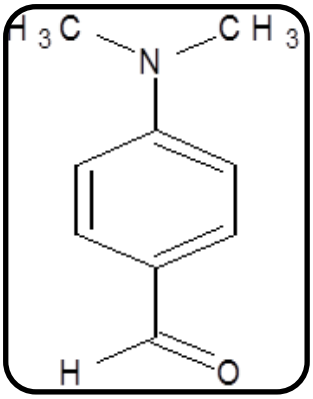
IUPAC NAME : anthracen-9-amine
Molecular Formula : $C_{14}H_{11}N$
Formula Weight : 300.20
Description : light purple
Melting point : 194 C
Solubility : ethanol

5- NITROFURALDEHYDE



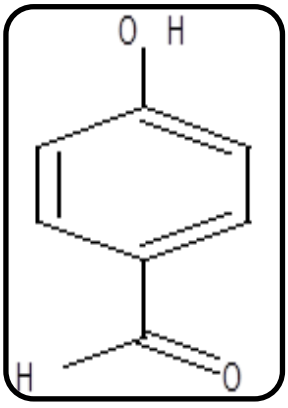
IUPAC NAME : 5-nitrofuran-2-carbaldehyde
Molecular Formula : $C_5H_3NO_5$
Formula Weight : 141.20
Description : light brow
Melting point : 39 C
Solubility : ethanol

PARA-DIMETHYLAMINOBENZALDEHYDE



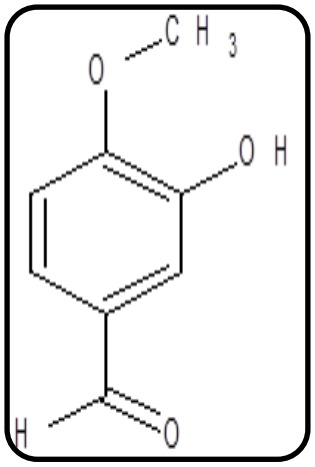
IUPAC NAME : 4-(dimethylamino)benzaldehyde
Molecular Formula : $C_9H_{11}NO$
Formula Weight : 149.20
Description : white color crystal
Melting point : 75 C
Solubility : ethanol

4-HYDROXY BENZADEHYDE



IUPAC NAME : 4-hydroxybenzaldehyde
Molecular Formula : $C_7H_6O_2$
Formula Weight : 122.20
Description : tan color powder
Melting point : 75 C
Solubility : ethanol

VANILINE



IUPAC NAME : 3-hydroxy-4-methoxybenzaldehyde

Molecular Formula : $C_8H_8O_3$

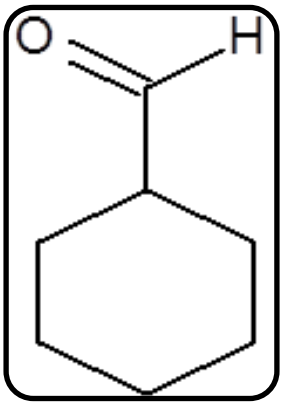
Formula Weight : 152.20

Description : white crystal

Melting point : 83 C

Solubility : ethanol

CYCLOHEXANECARBOXALDEHYDE



IUPAC NAME : cyclohexanecarbaldehyde

Molecular Formula : $C_7H_{12}O$

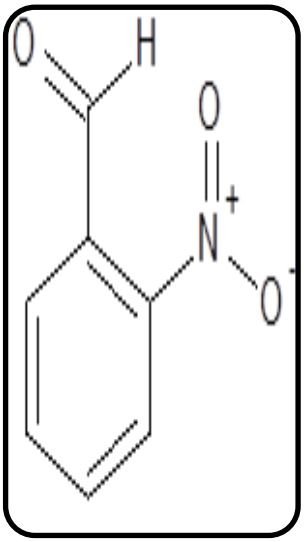
Formula Weight : 112.20

Description : colorless liquid

Melting point : 35 C

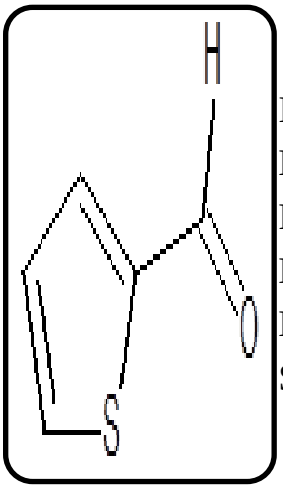
Solubility : ethanol

2 NITROBENZALDEHYDE



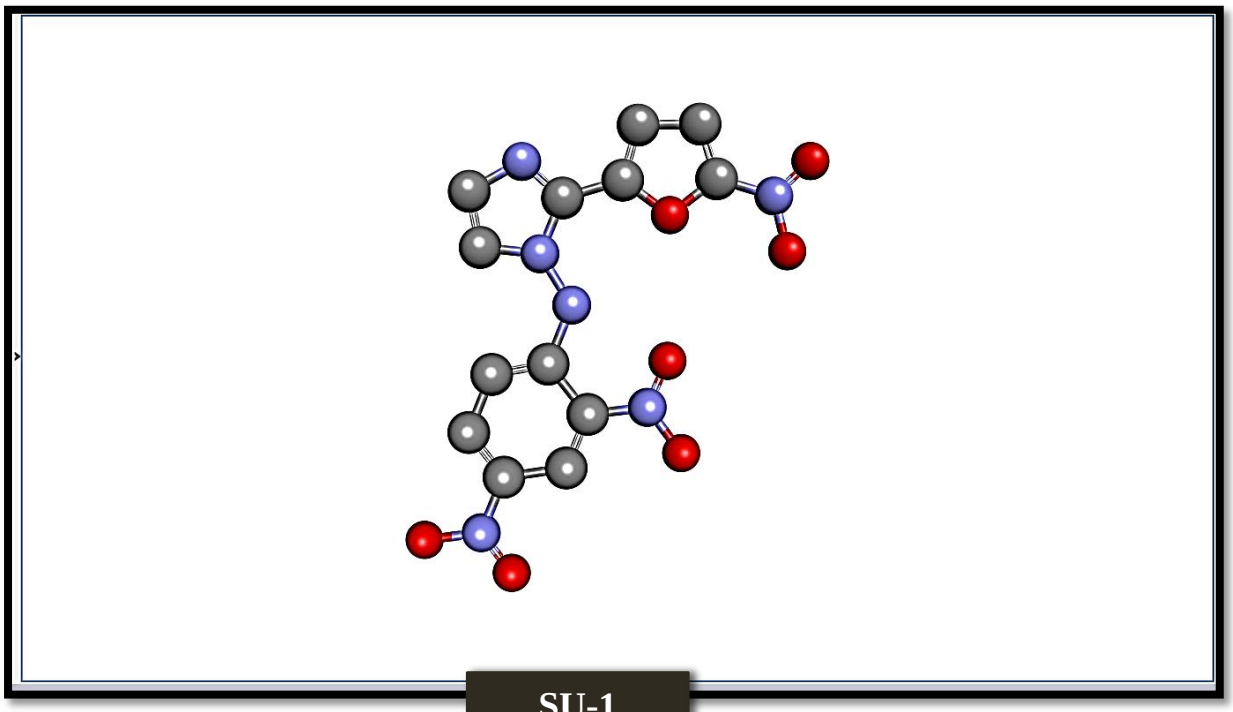
IUPAC NAME : 2-nitrobenzaldehyde
Molecular Formula : $C_7H_5NO_3$
Formula Weight : 151.20
Description : white color powder
Melting point : **43** C
Solubility : ethanol

THIOPHENECARBOXALDEHY



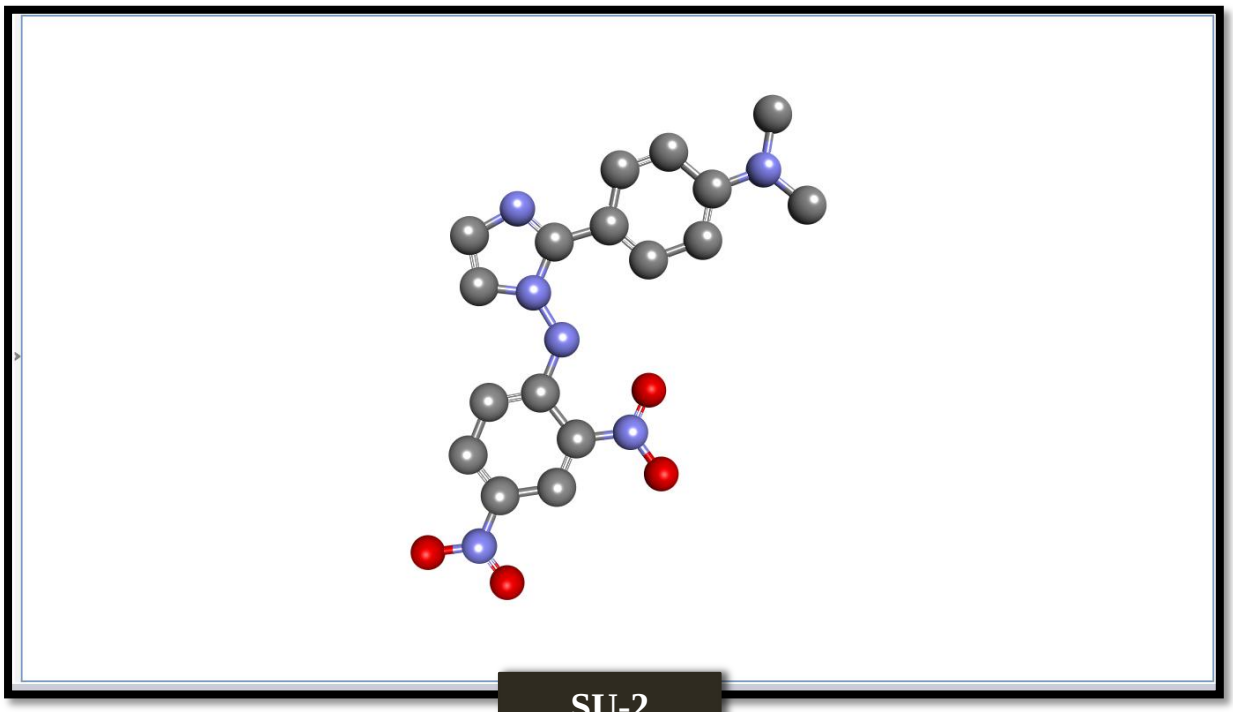
IUPAC NAME : thiophene-2-carbaldehyde
Molecular Formula : C_5H_4OS
Formula Weight : 112.20
Description : color less liquid
Melting point : **15** C
Solubility : ethanol

Product profile



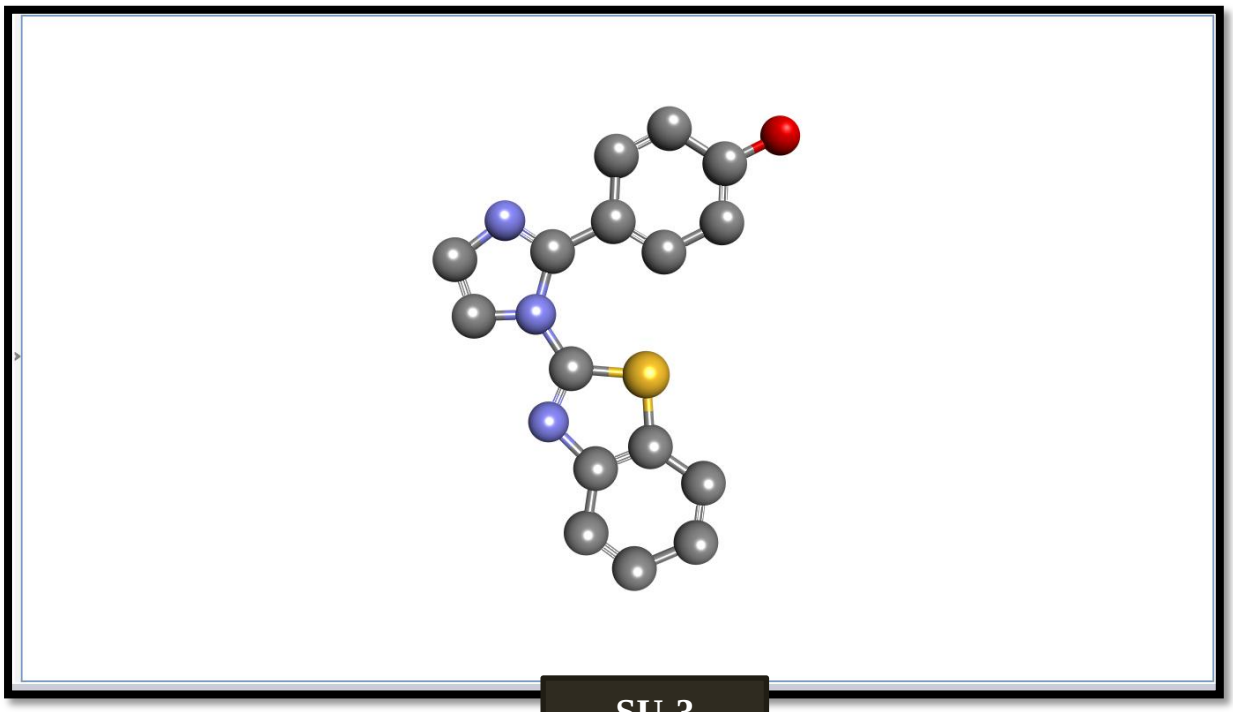
SU-1

- **Molecular Formula = $C_{13}H_8N_6O_7$**
- **Formula Weight = 360.23862**
- **Composition = C (43.34%) H(2.24%) N(23.33%) O(31.09%)**
- **Molar Refractivity = 83.24 ± 0.5 cm^3**
- **Molar Volume = 199.0 ± 7.0 cm^3**
- **Parachor = 621.0 ± 8.0 cm^3**
- **Index of Refraction = 1.776 ± 0.05**
- **Surface Tension = 94.7 ± 7.0 dyne/cm**
- **Density = 1.80 ± 0.1 g/cm^3**
- **Dielectric Constant = Not available**
- **Polarizabilitv = 33.00 ± 0.5 10^-**



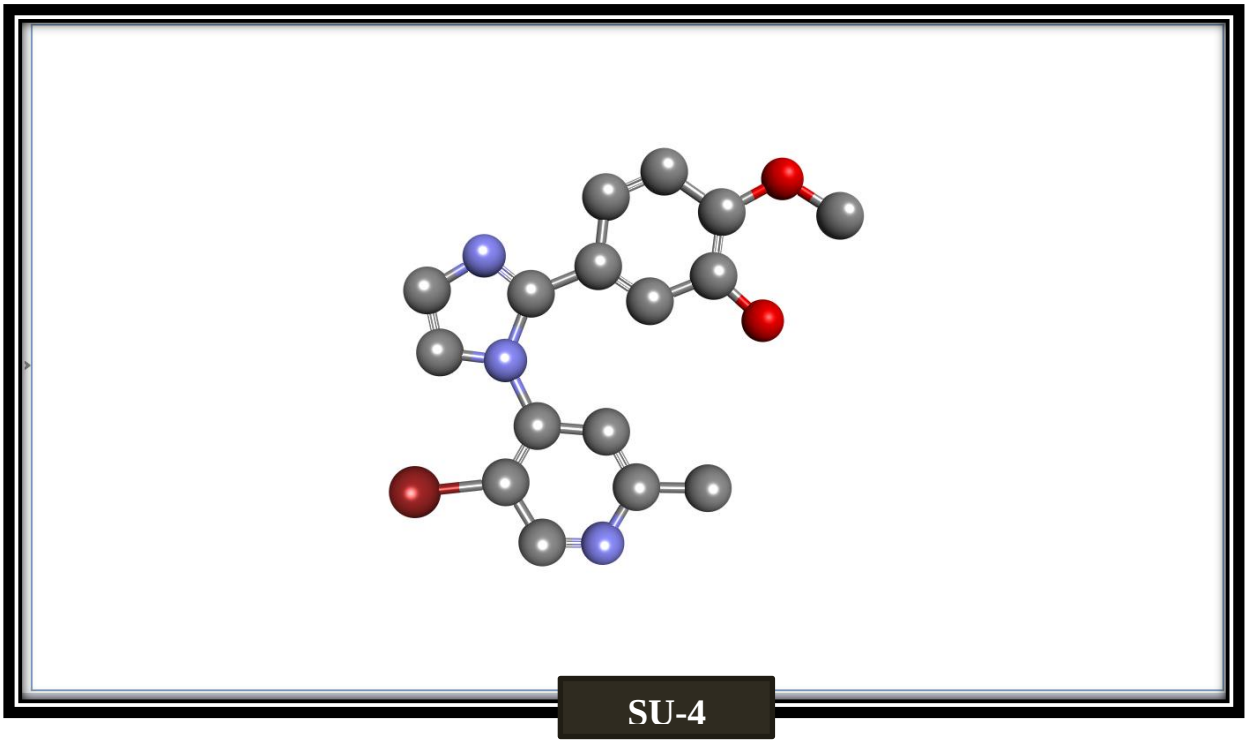
SU-2

- **Molecular Formula** = $C_{17}H_{16}N_6O_4$
- **Formula Weight** = 368.34674
- **Composition** = C(55.43%) H(4.38%) N(22.82%) O(17.37%)
- **Molar Refractivity** = 98.21 ± 0.5 cm^3
- **Molar Volume** = 260.6 ± 7.0 cm^3
- **Parachor** = 729.9 ± 8.0 cm^3
- **Index of Refraction** = 1.677 ± 0.05
- **Surface Tension** = 61.5 ± 7.0 dyne/cm
- **Density** = 1.41 ± 0.1 g/cm^3
- **Dielectric Constant** = Not available
- **Polarizability** = 38.93 ± 0.5 $10^{-24}cm^3$
- **Monoisotopic Mass** = 368.123303 Da



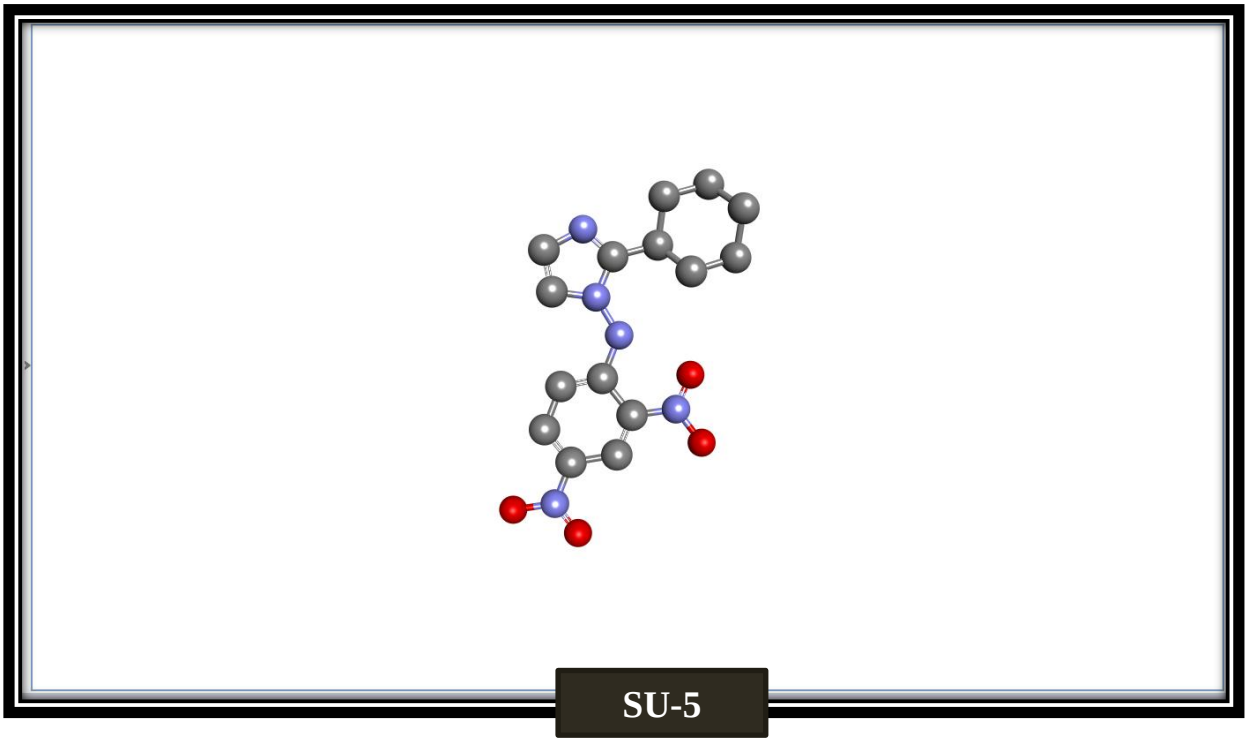
SU-3

- **Molecular Formula = $C_{16}H_{11}N_3OS$**
- **Formula Weight = 293.34304**
- **Composition = C(65.51%) H(3.78%) N(14.32%) O(5.45%) S(10.93%)**
- **Molar Refractivity = 84.81 ± 0.5 cm^3**
- **Molar Volume = 208.1 ± 7.0 cm^3**
- **Parachor = 581.7 ± 8.0 cm^3**
- **Index of Refraction = 1.750 ± 0.05**
- **Surface Tensio n = 61.0 ± 7.0 dyne/cm**
- **Density = 1.40 ± 0.1 g/cm^3**
- **Dielectric Constant = Not available**
- **Polarizability = 33.62 ± 0.5 $10^{-24}cm^3$**
- **Monoisotopic Mass = 293.062282 Da**



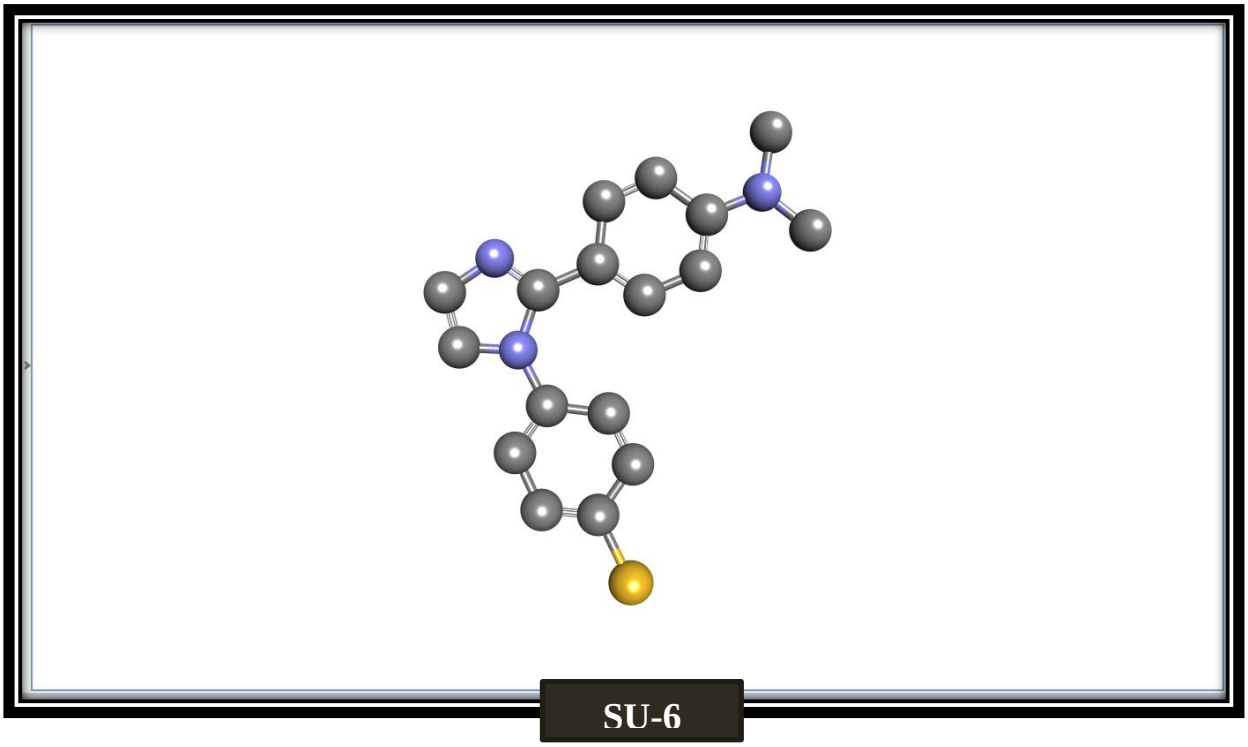
SU-4

- **Molecular Formula** = $C_{16}H_{14}BrN_3O_2$
- **Formula Weight** = 360.20526
- **Composition** = C(53.35%) H(3.92%) Br(22.18%) N(11.67%) O(8.88%)
- **Molar Refractivity** = 88.12 ± 0.5 cm^3
- **Molar Volume** = 239.4 ± 7.0 cm^3
- **Parachor** = 634.7 ± 8.0 cm^3
- **Index of Refraction** = 1.657 ± 0.05
- **Surface Tension** = 49.3 ± 7.0 dyne/cm
- **Density** = 1.50 ± 0.1 g/cm^3
- **Dielectric Constant** = Not available
- **Polarizability** = 34.93 ± 0.5 $10^{-24}cm^3$
- **Monoisotopic Mass** = 359.026932 Da



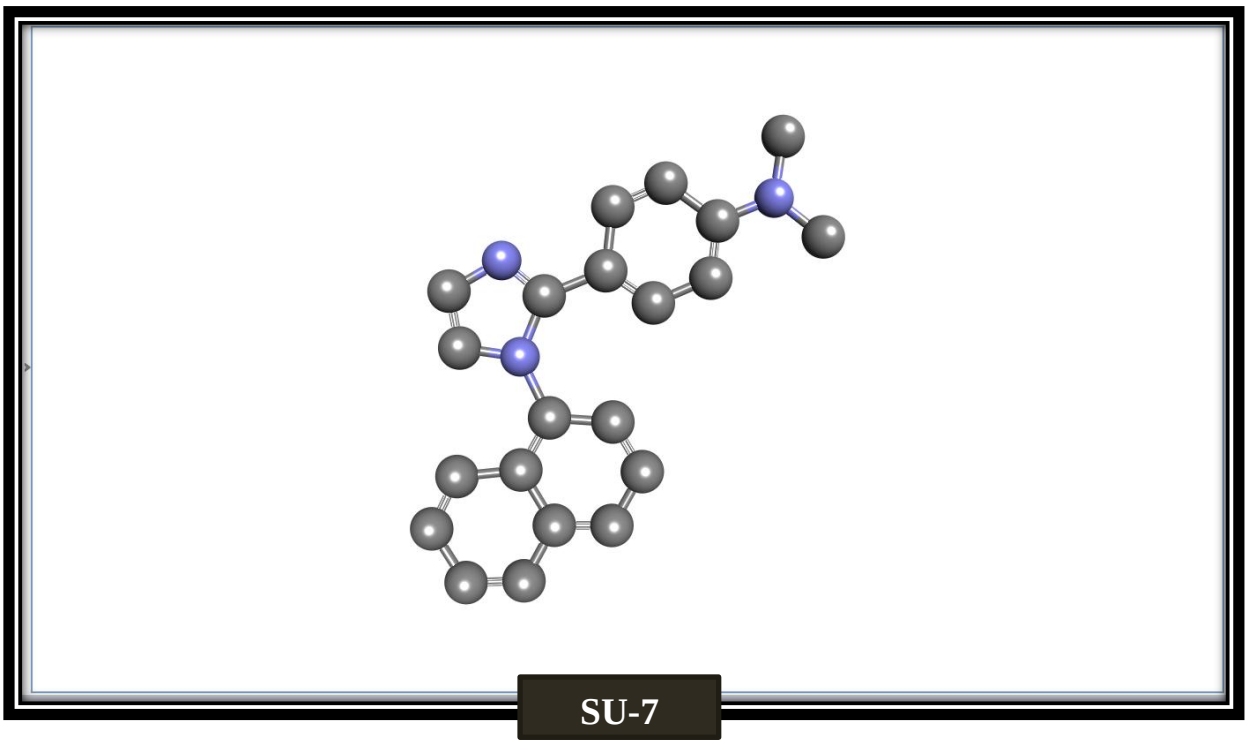
SU-5

- **Molecular Formula** = $C_{15}H_{17}N_5O_4$
- **Formula Weight** = 331.32658
- **Composition** = C(54.38%) H(5.17%) N(21.14%) O(19.32%)
- **Molar Refractivity** = 85.41 ± 0.5 cm^3
- **Molar Volume** = 219.4 ± 7.0 cm^3
- **Parachor** = 633.6 ± 8.0 cm^3
- **Index of Refraction** = 1.706 ± 0.05
- **Surface Tension** = 69.5 ± 7.0 dyne/cm
- **Density** = 1.50 ± 0.1 g/cm^3
- **Dielectric Constant** = Not available
- **Polarizability** = 33.85 ± 0.5 $10^{-24}cm^3$
- **Monoisotopic Mass** = 331.128054 Da



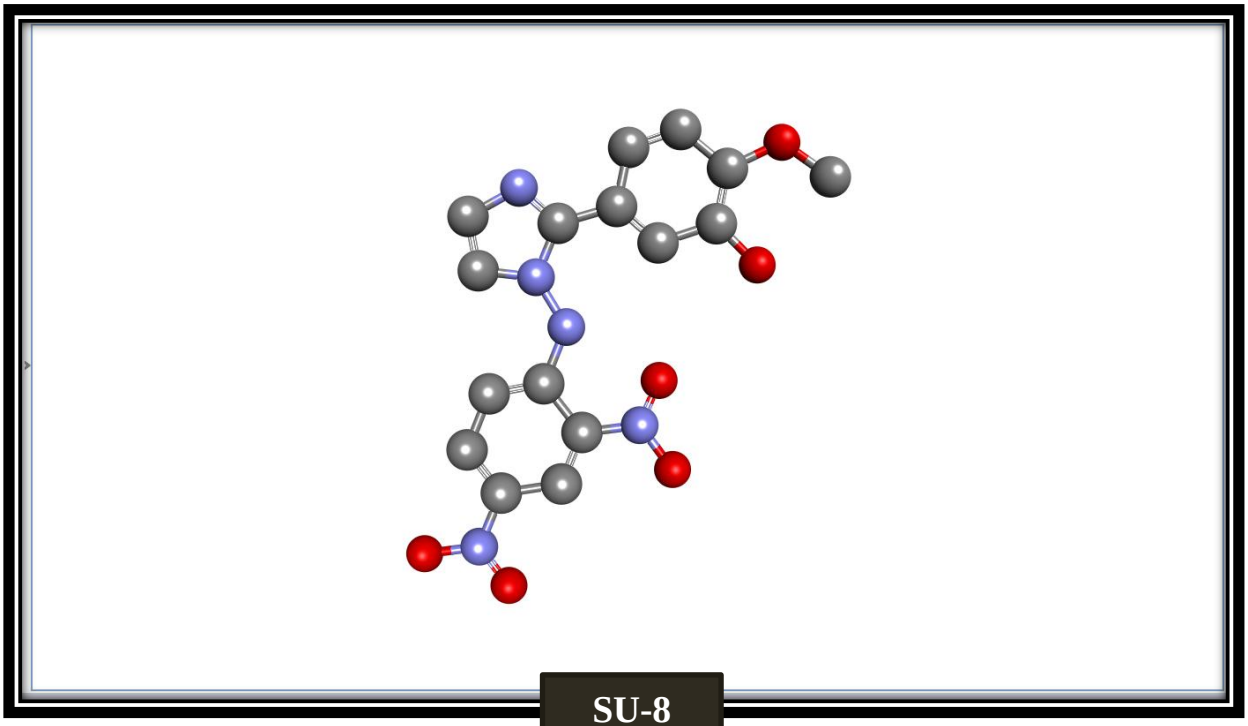
SU-6

- **Molecular Formula** = $C_{17}H_{17}N_3S$
- **Formula Weight** = 295.40198
- **Composition** = C(69.12%) H(5.80%) N(14.22%) S(10.85%)
- **Molar Refractivity** = 91.16 ± 0.5 cm^3
- **Molar Volume** = 253.9 ± 7.0 cm^3
- **Parachor** = 648.1 ± 8.0 cm^3
- **Index of Refraction** = 1.637 ± 0.05
- **Surface Tension** = 42.3 ± 7.0 dyne/cm
- **Density** = 1.16 ± 0.1 g/cm^3
- **Dielectric Constant** = Not available
- **Polarizability** = 36.14 ± 0.5 $10^{-24}cm^3$
- **Monoisotopic Mass** = 295.114318 Da



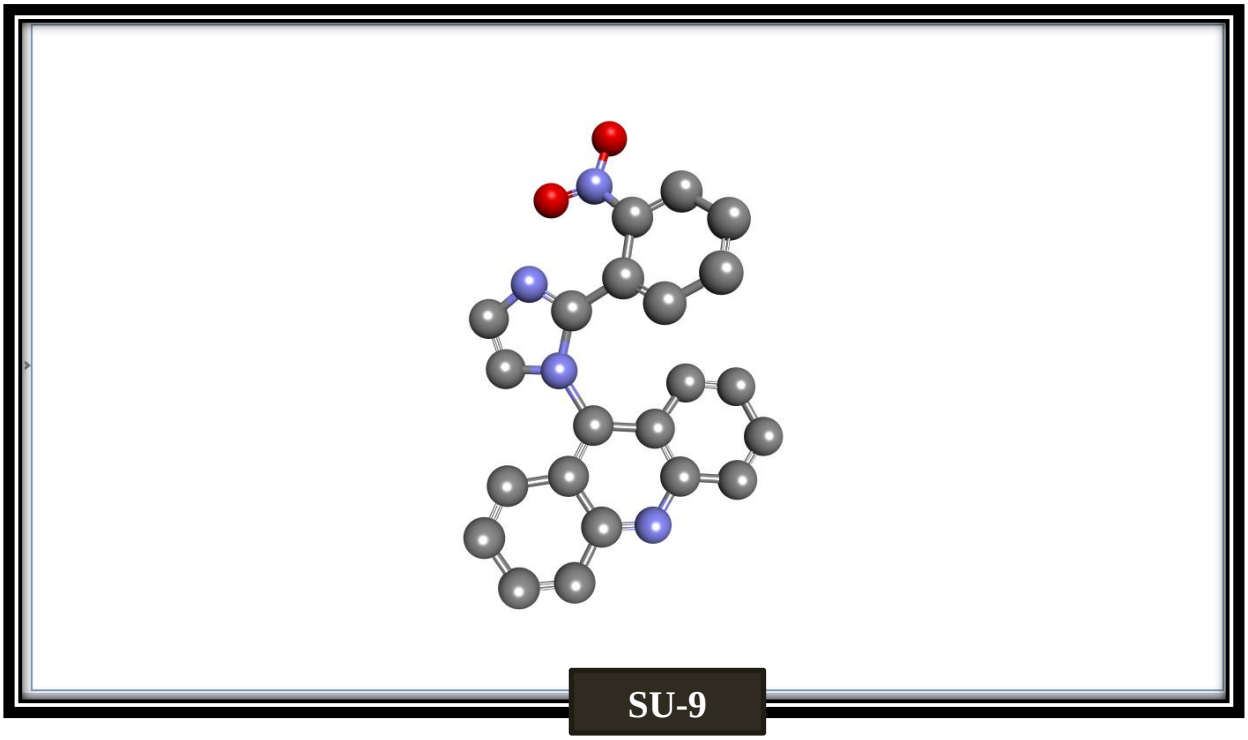
SU-7

- **Molecular Formula** = $C_{21}H_{19}N_3$
- **Formula Weight** = 313.39566
- **Composition** = C(80.48%) H(6.11%) N(13.41%)
- **Molar Refractivity** = 99.72 ± 0.5 cm^3
- **Molar Volume** = 281.0 ± 7.0 cm^3
- **Parachor** = 718.9 ± 8.0 cm^3
- **Index of Refraction** = 1.627 ± 0.05
- **Surface Tension** = 42.7 ± 7.0 dyne/cm
- **Density** = 1.11 ± 0.1 g/cm^3
- **Dielectric Constant** = Not available
- **Polarizability** = 39.53 ± 0.5 $10^{-24} cm^3$
- **Monoisotopic Mass** = 313.157898 Da



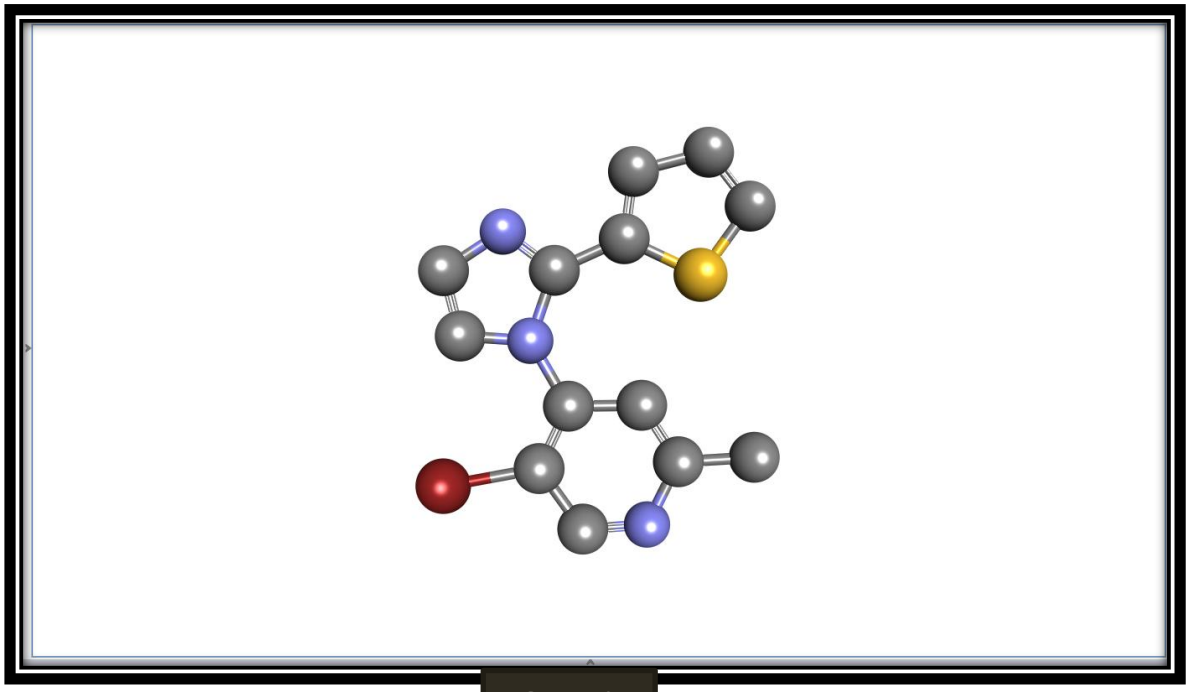
SU-8

- **Molecular Formula = $C_{16}H_{13}N_5O_6$**
- **Formula Weight = 371.30432**
- **Composition = C(51.76%) H(3.53%) N(18.86%) O(25.85%)**
- **Molar Refractivity = 92.07 ± 0.5 cm^3**
- **Molar Volume = 238.3 ± 7.0 cm^3**
- **Parachor = 689.6 ± 8.0 cm^3**
- **Index of Refraction = 1.699 ± 0.05**
- **Surface Tension = 70.0 ± 7.0 dyne/cm**
- **Density = 1.55 ± 0.1 g/cm^3**
- **Dielectric Constant = Not available**
- **Polarizability = 36.50 ± 0.5 $10^{-24}cm^3$**
- **Monoisotopic Mass = 371.086583 Da**



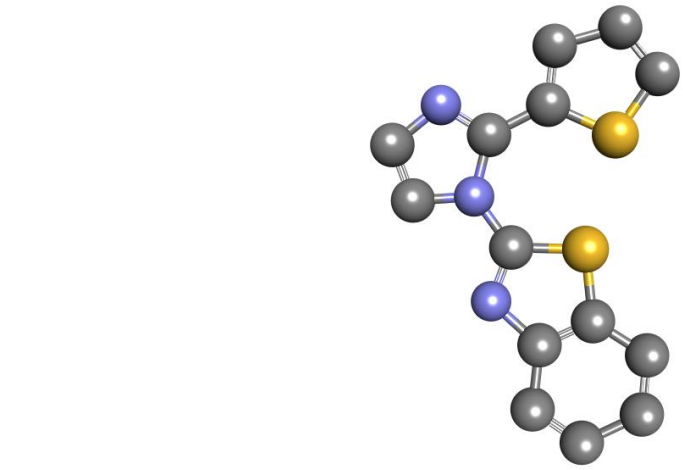
SU-9

- **Molecular Formula** = $C_{22}H_{14}N_4O_2$
- **Formula Weight** = 366.37216
- **Composition** = C(72.12%) H(3.85%) N(15.29%) O(8.73%)
- **Molar Refractivity** = 106.91 ± 0.5 cm^3
- **Molar Volume** = 269.5 ± 7.0 cm^3
- **Parachor** = 748.8 ± 8.0 cm^3
- **Index of Refraction** = 1.723 ± 0.05
- **Surface Tension** = 59.5 ± 7.0 dyne/cm
- **Density** = 1.35 ± 0.1 g/cm^3
- **Dielectric Constant** = Not available
- **Polarizability** = 42.38 ± 0.5 $10^{-24}cm^3$
- **Monoisotopic Mass** = 366.111676 Da



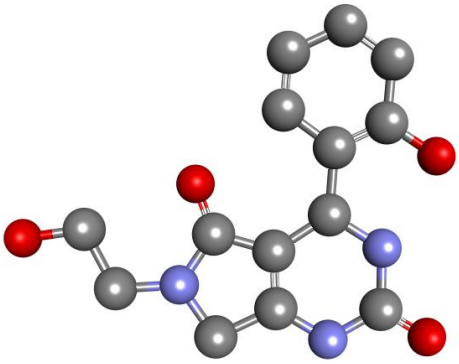
SU-10

- **Molecular Formula = $C_{13}H_{10}BrN_3S$**
- **Formula Weight = 320.2076**
- **Composition = C(48.76%) H(3.15%) Br(24.95%) N(13.12%) S(10.01%)**
- **Molar Refractivity = 80.05 ± 0.5 cm^3**
- **Molar Volume = 202.7 ± 7.0 cm^3**
- **Parachor = 551.0 ± 8.0 cm^3**
- **Index of Refraction = 1.719 ± 0.05**
- **Surface Tension = 54.5 ± 7.0 dyne/cm**
- **Density = 1.57 ± 0.1 g/cm^3**
- **Dielectric Constant = Not available**
- **Polarizability = 31.73 ± 0.5 $10^{-24}cm^3$**
- **Monoisotopic Mass = 318.977872 Da**



SU-11

- **Molecular Formula** = $C_{14}H_9N_3S_2$
- **Formula Weight** = 283.37136
- **Composition** = C(59.34%) H(3.20%) N(14.83%) S(22.63%)
- **Molar Refractivity** = 82.55 ± 0.5 cm^3
- **Molar Volume** = 193.1 ± 7.0 cm^3
- **Parachor** = 548.3 ± 8.0 cm^3
- **Index of Refraction** = 1.799 ± 0.05
- **Surface Tension** = 64.9 ± 7.0 dyne/cm
- **Density** = 1.46 ± 0.1 g/cm^3
- **Dielectric Constant** = Not available
- **Polarizability** = 32.72 ± 0.5 $10^{-24}cm^3$
- **Monoisotopic Mass** = 283.023787 Da



SU-12

- **Molecular Formula** = $C_{14}H_{13}N_3O_4$
- **Formula Weight** = 287.27072
- **Composition** = C(58.53%) H(4.56%) N(14.63%) O(22.28%)
- **Molar Refractivity** = 72.88 ± 0.5 cm^3
- **Molar Volume** = 181.8 ± 7.0 cm^3
- **Parachor** = 526.8 ± 8.0 cm^3
- **Index of Refraction** = 1.734 ± 0.05
- **Surface Tension** = 70.4 ± 7.0 dyne/cm
- **Density** = 1.57 ± 0.1 g/cm^3
- **Dielectric Constant** = Not available
- **Polarizability** = 28.89 ± 0.5 $10^{-24}cm^3$
- **Monoisotopic Mass** = 287.090606 Da

Results and discussion

RESULT AND DISCUSSION

Already we discussed about the design and synthesis in materials an methods. Now we discuss about the result of Characterisation and in-vitro evaluation of synthesized compounds

Physical properties of the synthesized compounds

A) **Melting point, Percentage yield.**-Table-5

Compounds	Melting Point ^{O}C	% Yield
SU1	152-154	85 %
SU2	148-150	82 %
SU3	160-162	83 %
SU4	150-152	86 %
SU5	154-156	86 %
SU6	158-160	84 %
SU7	140-142	84 %
SU8	170-172	82 %
SU9	146-148	83 %
SU10	156-158	82 %
SU11	142-144	80%
SU12	174-176	81%

B) TLC profile- Table-6

Compounds	R_f	Solvent System
SU1	0.42	Ethyl acetate: hexane (6:4)
SU2	0.37	Ethyl acetate: hexane (6:4)
SU3	0.38	Ethyl acetate: hexane (6:4)
SU4	0.35	Ethyl acetate: hexane (6:4)
SU5	0.27	Ethyl acetate: hexane (6:4)
SU6	0.28	Ethyl acetate: hexane (6:4)
SU7	0.39	Ethyl acetate: hexane (6:4)
SU8	0.25	Ethyl acetate: hexane (6:4)
SU9	0.30	Ethyl acetate: hexane (6:4)
SU10	0.26	Ethyl acetate: hexane (6:4)
SU11	0.24	Ethyl acetate: hexane (6:4)
SU12	0.31	Ethyl acetate: hexane (6:4)

.

C) Solubility- Table-7

Compounds	Water	Methanol	Ethanol	Ethyl acetate	DMSO	DMF
SU1	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU2	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU3	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU4	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU5	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU6	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU7	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU8	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU9	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU10	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU11	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
SU12	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble

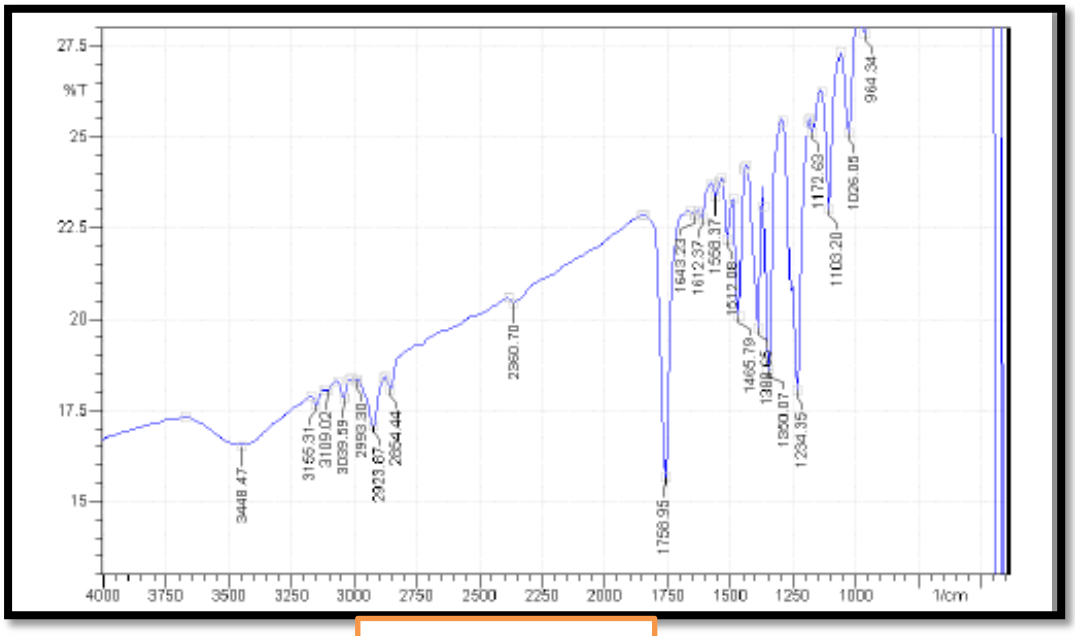
The following experimental methods were used for the characterization of the synthesized compounds.

1. Infra-Red Spectroscopy by Perkin Elmer FTIR Spectrometer using KBr pellets.
2. ^{1}H and ^{13}C Nuclear Magnetic Spectroscopy by 500 MHZ Jeol using DMSO.
3. The Mass Spectroscopy by Jeol GC mate.
4. Melting Points by Open Capillary Tubes.

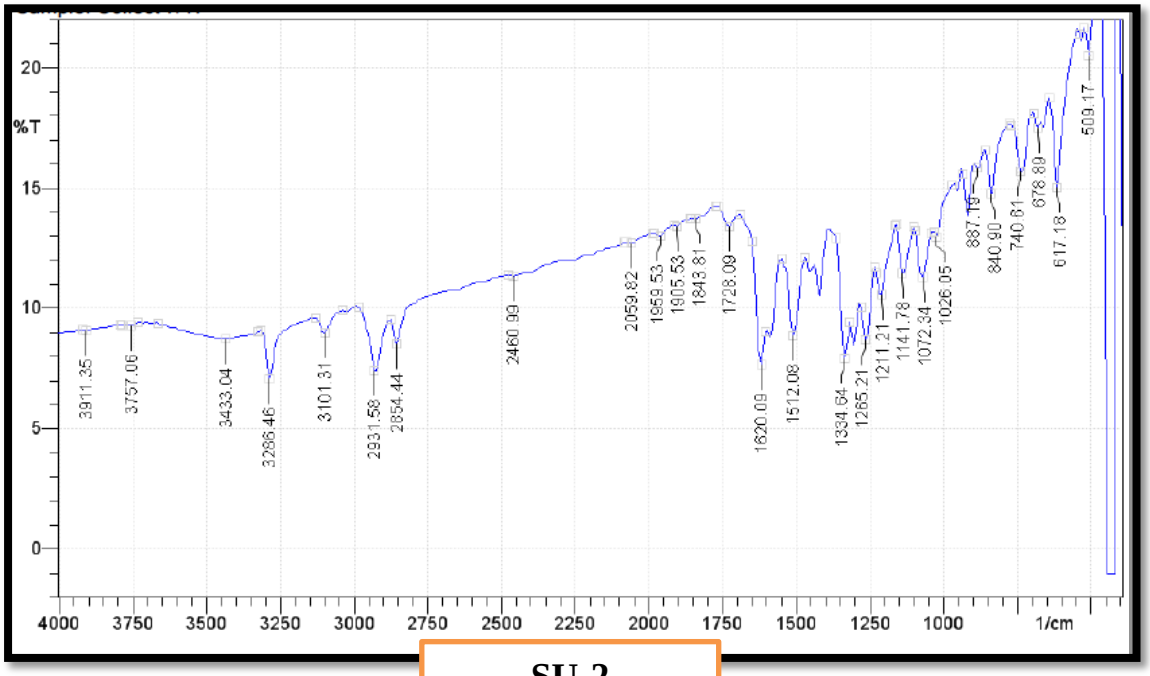
A) **IR spectral data of the synthesized compounds-** Table-8

Compounds	IR Absorption Region (cm^{-1})
SU1	3338.64 cm^{-1} (Ar-CH), 1519.47 cm^{-} (C=C), 1152.16 cm^{-1} (C-N), 1022.75 cm^{-1} (C=C-O-C).
SU2	3308.37 cm^{-1} 3056.69 cm^{-1} (Ar-CH), 1521.42 cm^{-1} (C=C), 1154.74 cm^{-1} (C-N), 1016.04 cm^{-1} (C=C-O-C), 739.57 cm^{-1} (C-Cl).
SU3	3309.63 cm^{-1}, 3055.53 cm^{-1} (Ar-CH), 1522.76 cm^{-1} (C=C), 1155.74 cm^{-1} (C-N), 1012.84 cm^{-1} (C=C-O-C), 578.63 cm^{-1} (C-Br).
SU4	3060.70 cm^{-1} (Ar-CH),), 1509.22 cm^{-1} (C=C), 1155.29 cm^{-1} (C-N), 1012.93 cm^{-1} (C=C-O-C), 1409.17 cm^{-1} (C-F).
SU5	3052.13 cm^{-1} (Ar-CH), 1523.35 cm^{-1} (C=C), 1150.04 cm^{-1} (C-N), 1037.99 cm^{-1} (C=C-O-C), 745.21 cm^{-1} (C-Cl).
SU6	3064.5 cm^{-1} (Ar-CH), 1523.72 cm^{-1} (C=C), 1404.00 cm^{-1} (C-F), 1152.0 cm^{-1} (C-N), 1020.48 cm^{-1} (C=C-O-C).
SU7	3053.82 cm^{-1} (Ar-CH), 1518.98 cm^{-1} (C=C), 1153.95 cm^{-1} (C-N), 1020.96 cm^{-1} (C=C-O-C), 575.55 cm^{-1} (C-Br).
SU8	3060.14 cm^{-1} (Ar-CH), 1527.62 cm^{-1} (C-NO_2), 1406.84 cm^{-1} (C=C), 1164.06 cm^{-1} (C-N), 1019.68 cm^{-1} (C=C-O-C).
SU9	3046.07 cm^{-1} (Ar-CH), 2924.41 cm^{-1} (Ar-CH_3), 2201.20 cm^{-1} (C≡N), 1522.38 cm^{-1} (C=C), 1150.70 cm^{-1} (C-N), 1022.18 cm^{-1} (C=C-O-C).
SU10	3060.46 cm^{-1} (Ar-CH), 1512.94 cm^{-1} (C=C), 1250.18 cm^{-1} (O-CH_3), 1152.03 cm^{-1} (C-N), 1021.87 cm^{-1} (C=C-O-C).
SU11	3046.07 cm^{-1} (Ar-CH), 1514.47 cm^{-1} (C=C), 1264.32 cm^{-1} (O-CH_3), 1151.01 cm^{-1} (C-N), 1023.44 cm^{-1} (C=C-O-C).
SU12	3055.53 cm^{-1} (Ar-CH), 1509.45 cm^{-1} (C=C), 1250.17 cm^{-1} (O-CH_3), 1155.15 cm^{-1} (C-N), 1025.17 cm^{-1} (C=C-O-C), 582.14 cm^{-1} (C-Br).

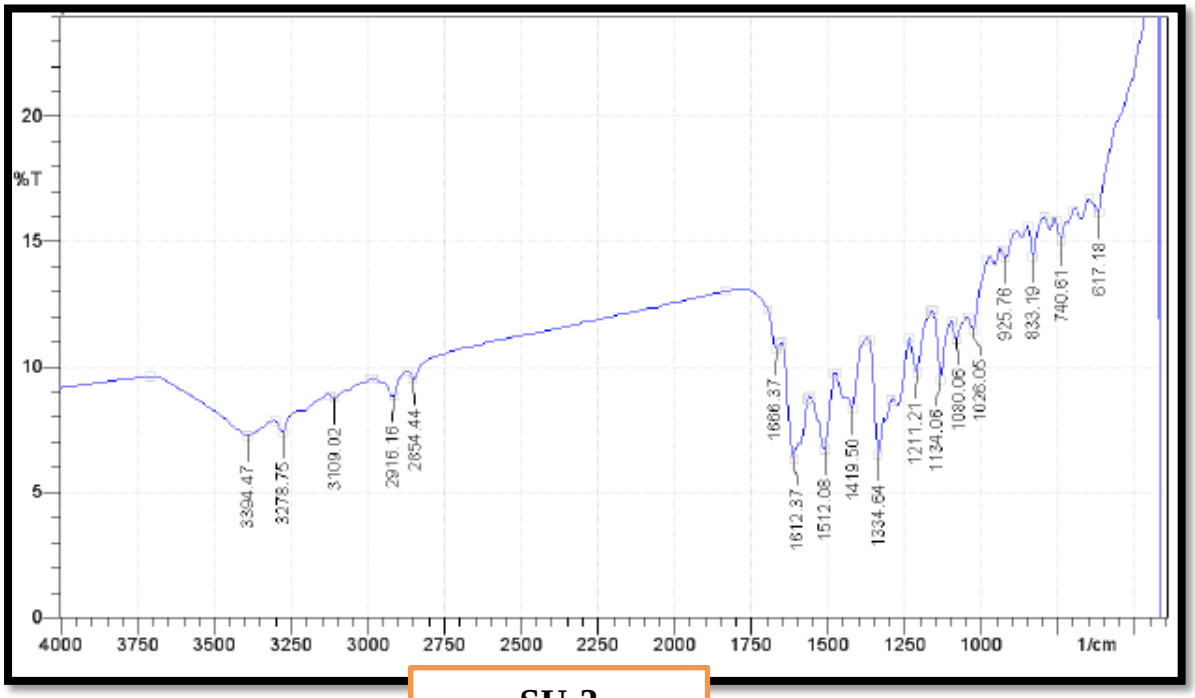
IR spectrum of the synthesized compounds



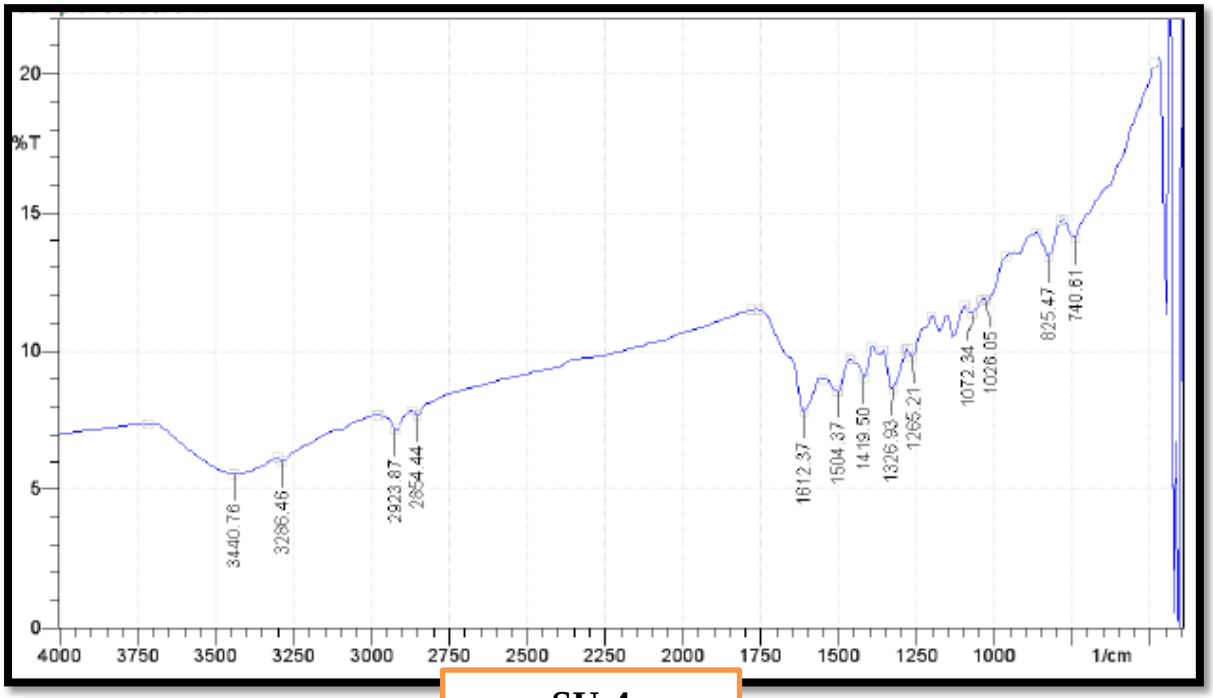
SU-1



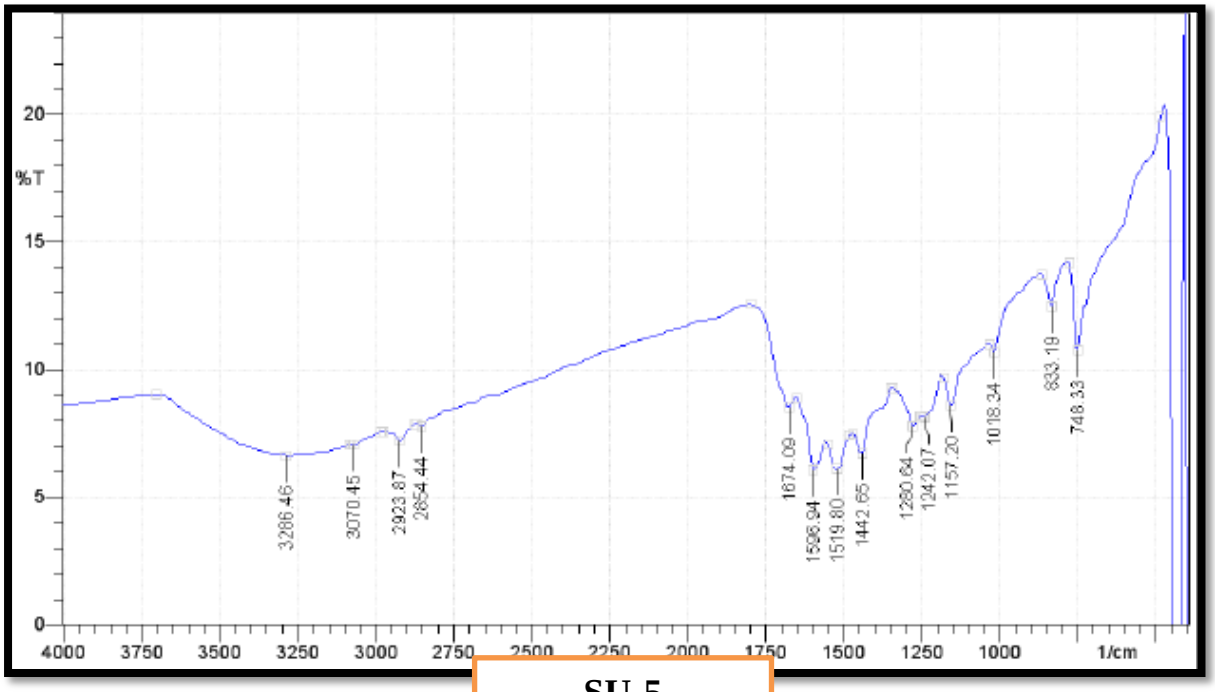
SU-2


%T
20
15
10
5
0
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm
3394.47
3278.75
3109.02
2916.16
2854.44
1666.37
1612.37
1512.08
1419.50
1334.64
1211.21
1134.06
1080.06
1026.05
925.76
833.19
740.61
617.18

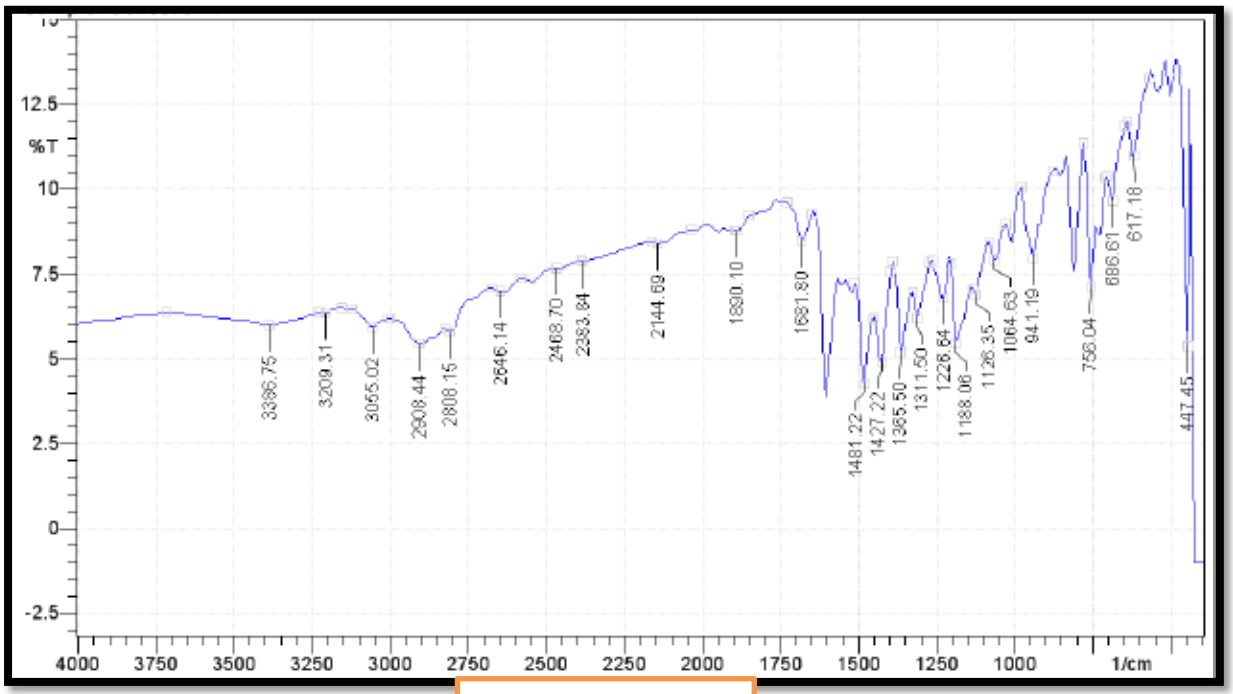
SU-3


%T
20
15
10
5
0
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm
3440.76
3286.46
2923.87
2854.44
1612.37
1504.37
1419.50
1326.93
1265.21
1072.34
1026.05
825.47
740.61

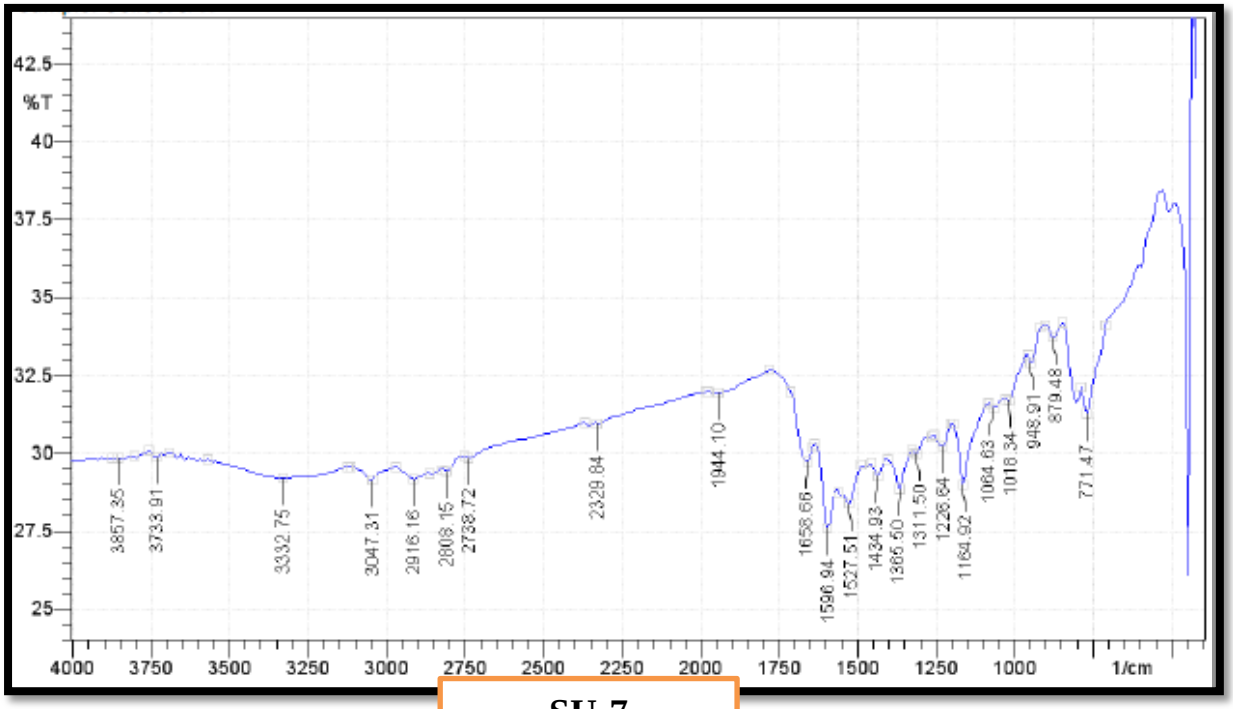
SU-4


%T
20
15
10
5
0
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm
3286.46
3070.45
2923.87
2854.44
1674.09
1596.94
1519.80
1442.65
1280.64
1242.07
1157.20
1018.34
833.19
748.33

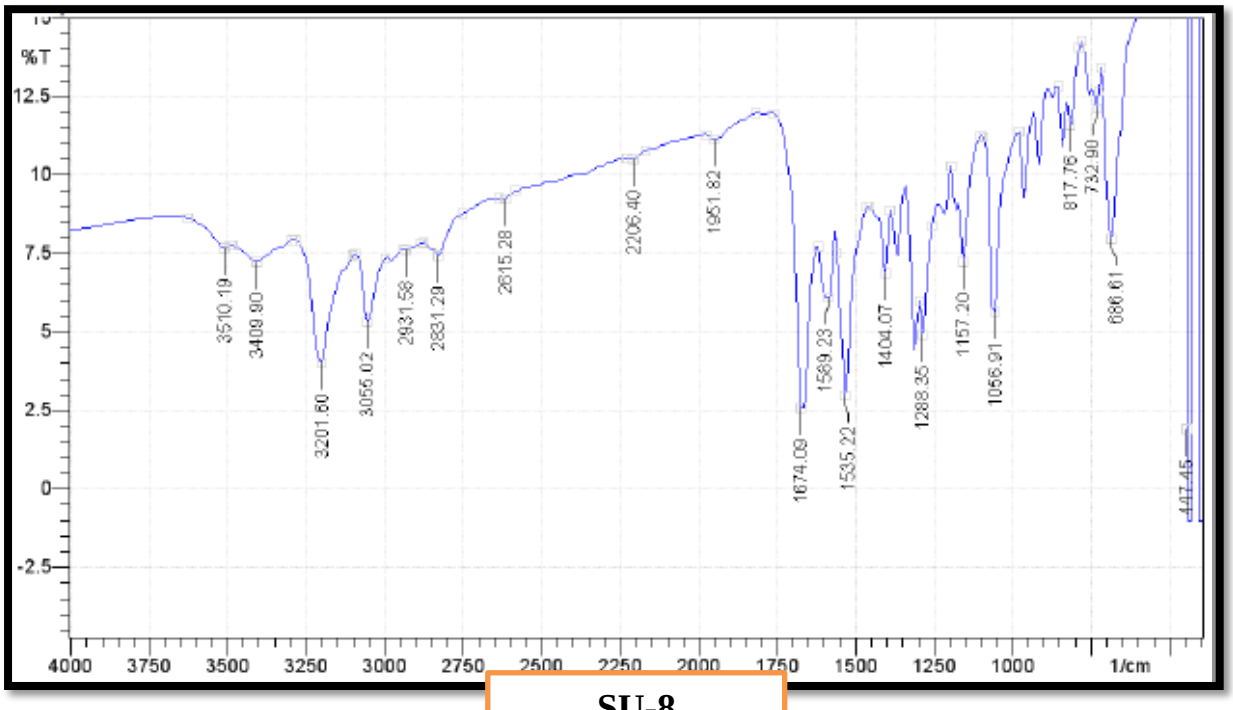
SU-5


%T
12.5
10
7.5
5
2.5
0
-2.5
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm
3386.75
3209.31
3055.02
2908.44
2808.15
2646.14
2468.70
2383.84
2144.69
1890.10
1681.80
1481.22
1427.22
1365.50
1311.50
1226.64
1188.06
1126.35
1064.63
941.19
758.04
686.61
617.18
447.45

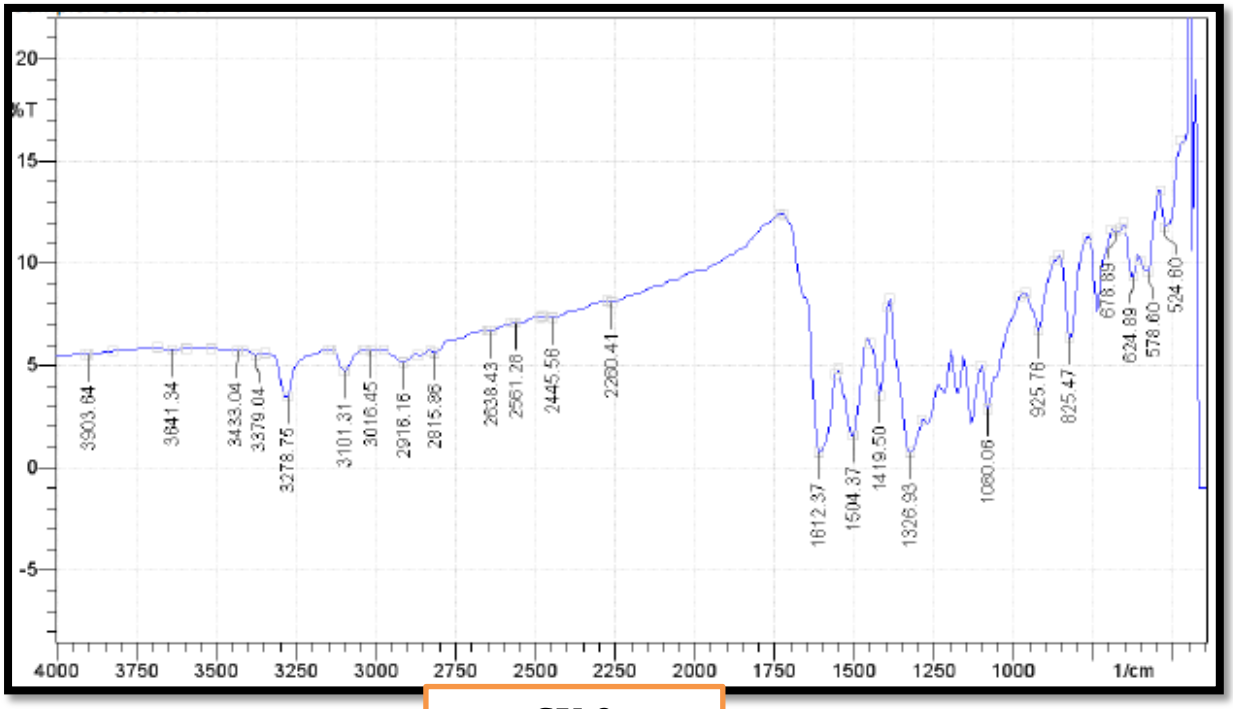
SU-6


%T
42.5
40
37.5
35
32.5
30
27.5
25
3857.35
3733.91
3332.75
3047.31
2916.16
2806.15
2738.72
2329.84
1944.10
1658.66
1596.94
1527.51
1434.93
1365.60
1311.50
1226.64
1164.92
1064.63
1018.34
948.91
879.48
771.47
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm

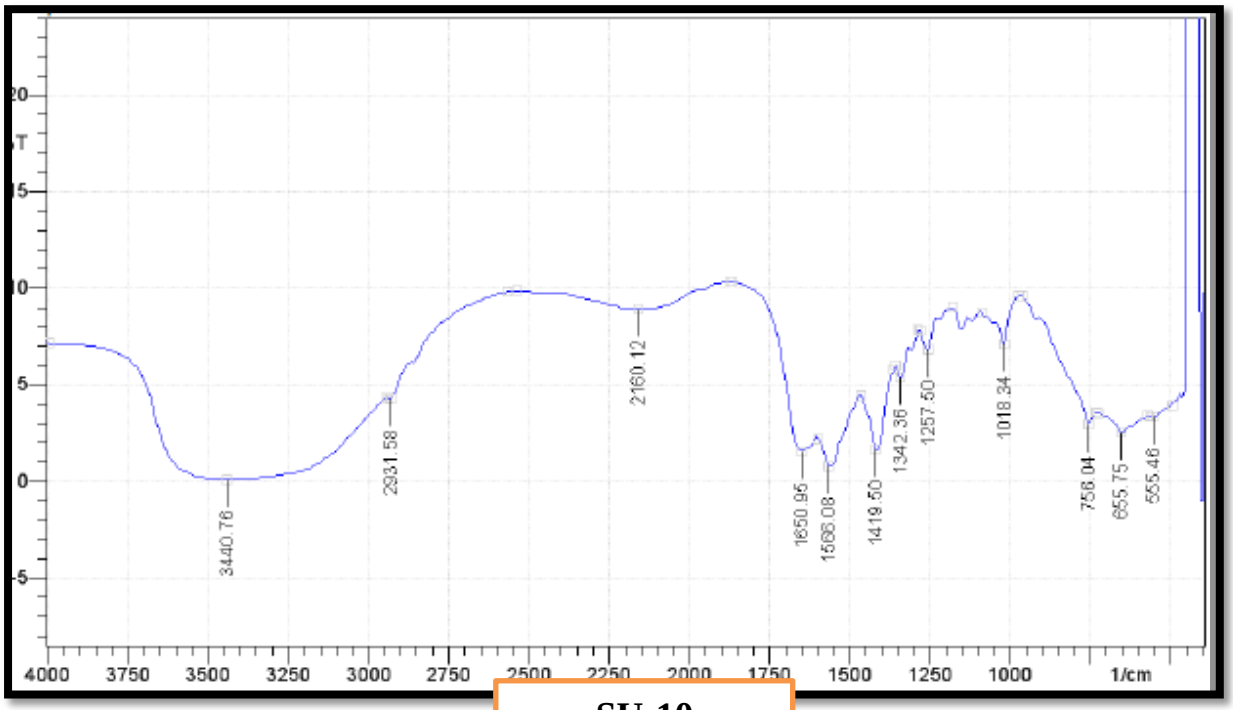
SU-7


%T
12.5
10
7.5
5
2.5
0
-2.5
3510.19
3409.90
3201.60
3056.02
2931.58
2831.29
2615.28
2206.40
1951.82
1674.09
1589.23
1535.22
1404.07
1288.35
1157.20
1056.91
817.76
732.90
686.61
447.45
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm

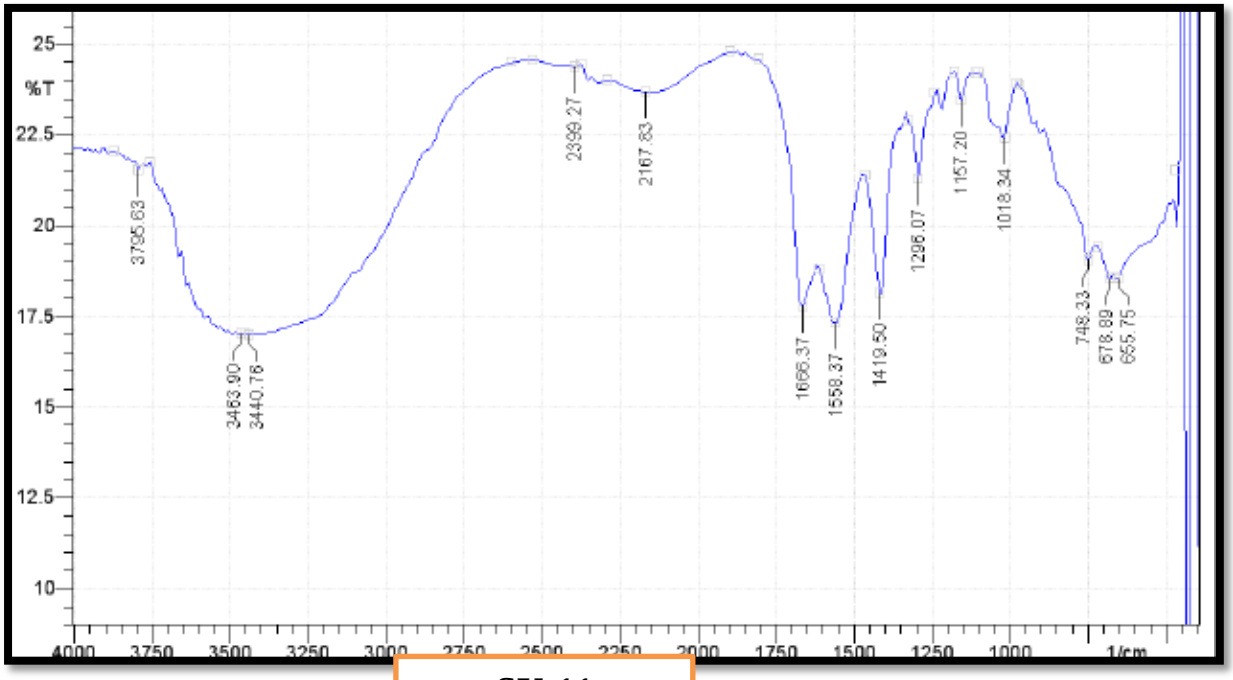
SU-8


%T
20
15
10
5
0
-5
3903.84
3841.34
3433.04
3379.04
3278.75
3101.31
3016.45
2916.16
2815.86
2638.43
2561.28
2445.56
2260.41
1812.37
1504.37
1419.50
1326.93
1060.06
925.76
825.47
676.89
624.89
578.60
524.60
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm

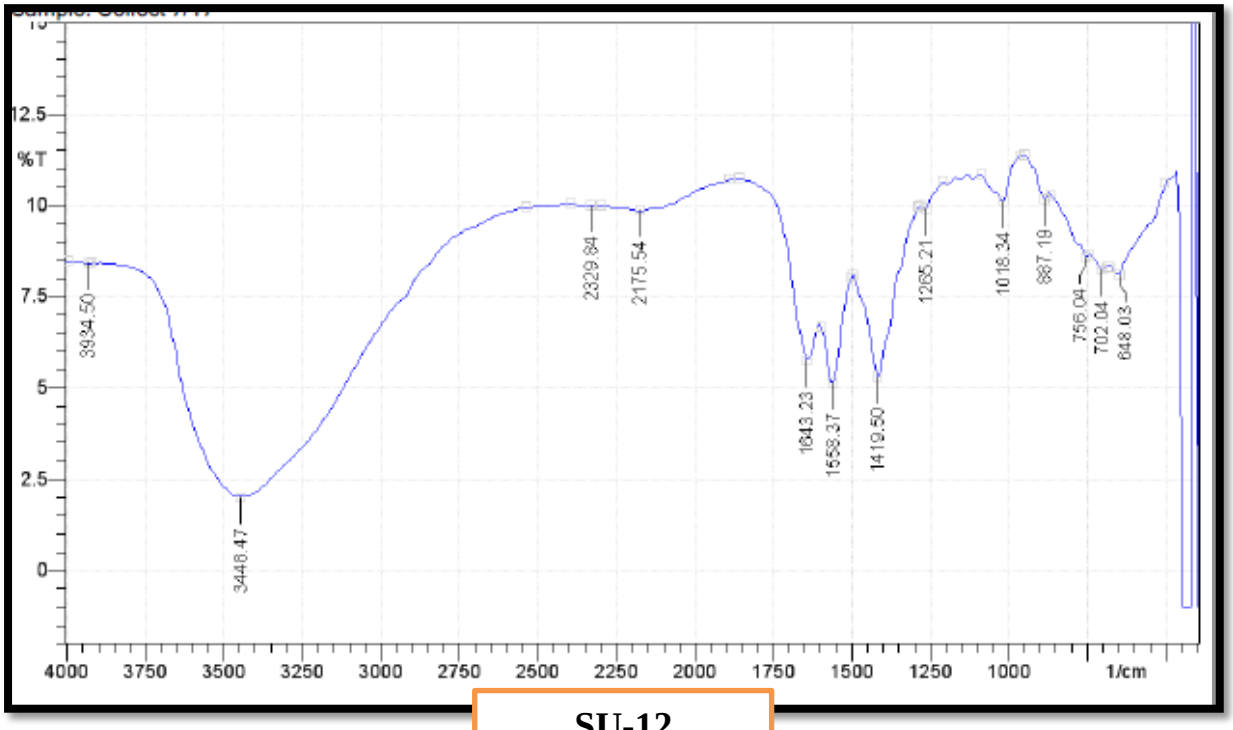
SU-9


3440.76
2931.58
2160.12
1650.95
1586.08
1419.50
1342.36
1257.50
1018.34
756.04
655.75
555.46
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
1/cm

SU-10


%T
3795.63
3463.90
3440.78
2399.27
2167.83
1666.37
1558.37
1419.50
1296.07
1157.20
1018.34
748.33
678.89
655.75
1/cm

SU-11


%T
3934.50
3448.47
2329.84
2175.54
1643.23
1558.37
1419.50
1265.21
1018.34
887.19
756.04
702.04
648.03
1/cm

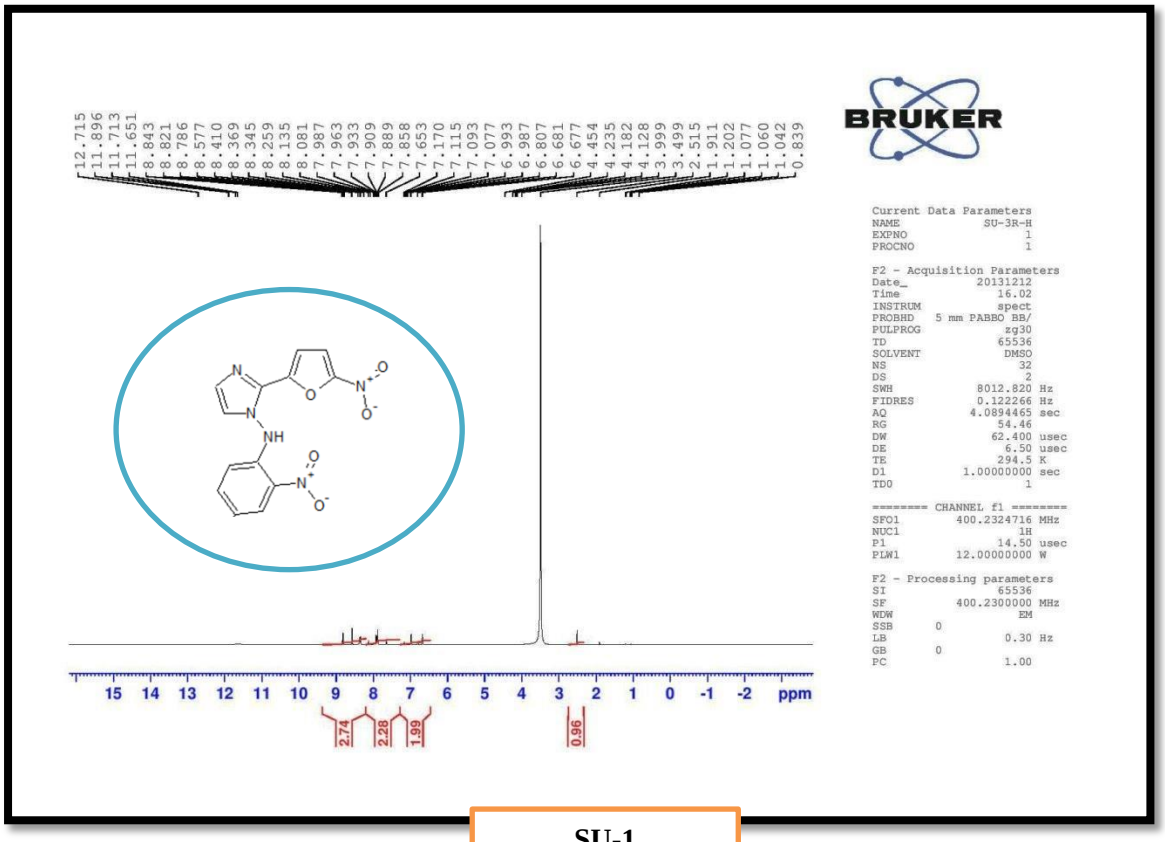
SU-12

A) ^{1}H NMR Spectral Data of the Synthesized Compounds- Table-9

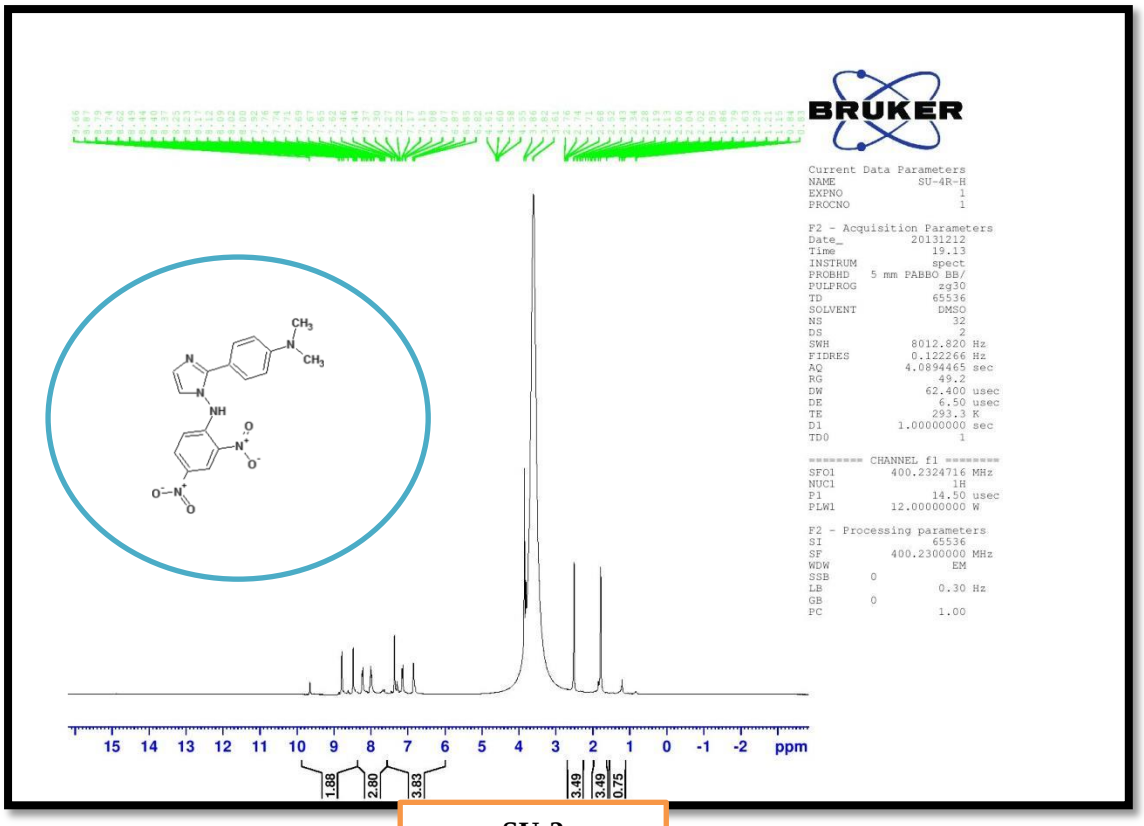
Compounds	^{1}H NMR Data (500 MHz, DMSO-d_6)
SU1	δ 4.40 (s, 1H, -CH), 7.14 (t , 1H, *J* = 6.9 Hz, Ar-H), 7.21 (m, 2H, Ar-H), 7.26 (s, 2H, -), 7.28-7.32 (m, 2H, Ar-H), 7.38-7.41 (m, 2H, Ar-H), 7.4 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.93 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.11 (d, 1H, *J* = 7.35 Hz, Ar-H), 11.96 (br s, 1H, -NH,)
SU2	δ 4.46 (s, 1H, -CH), 7.13 (t , 1H, *J* = 6.85 Hz, Ar-H), 7.20 (t, 1H, *J* = 7.65 Hz, Ar-H), 7.30 (s, 2H, -), 7.34-7.36 (m, 2H, Ar-H), 7.45-7.48 (m, 3H, Ar-H), 7.92 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.11 (d, 1H, *J* = 7.6 Hz, Ar-H), 11.96 (br s, 1H, -NH, -
SU3	δ 4.44 (s, 1H, -CH), 7.13 (t, 1H, *J* = 6.85 Hz, Ar-H), 7.20 (t, 1H, *J* = 7.65 Hz, Ar-H), 7.28 (s, 2H, -NH_2,- 7.30 (m, 2H, Ar-H), 7.47 (d, 1H, *J* = 8.45 Hz, Ar-H), 7.59 (m, 2H, Ar-H), 7.91 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.10 (d, 1H, *J* = 6.9 Hz, Ar-H), 11.9 (br s, 1H, -NH),
SU4	δ 4.45 (s, 1H, -CH), 7.13 (t, 1H, *J* – 7.3 Hz, Ar-H), 7.19-7.23 (m, 3H, Ar-H),7.27 (s, 2H, - 7.35-7.37 (m, 2H, Ar-H), 7.47 (d, 1H, *J* = 8.55 Hz, Ar-H), 7.92 (d, 1H, *J* = 8.55 Hz, Ar-H), 8.10 (d, 1H, *J* = 7.85 Hz, Ar-H), 11.95 (br s, 1H, -NH,)
SU5	δ 4.87 (s, 1H, -CH), 7.13 (t, 1H, *J* = 7.65 Hz, Ar-H), 7.20 (t, 1H, *J* = 7.65 Hz, Ar-H), 7.31 (s, 2H), 7.33-7.48 (m, 5H, Ar-H), 7.91 (d, 1H, *J* = 7.65 Hz, Ar-H), 8.09 (d, 1H, *J* = 7.7 Hz, Ar-H), 11.97 (br s, 1H, -NH,)
SU6	δ 4.66 (s, 1H, -CH), 7.13 (t, 1H, *J* = 6.85 Hz, Ar-H), 7.19-7.25 (m, 5H, Ar-H), 7.32 (s, 2H, 7.48 (d, 1H, *J* = 7.65 Hz, Ar-H), 7.92 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.11 (d, 1H, *J* = 6.9 Hz, Ar-H), 11.9 (br s, 1H, -NH,
SU7	δ 4.47 (s, 1H, -CH), 7.13 (t, 1H, J = 6.9 Hz, Ar-H), 7.20 (t, 1H, J = 7.65 Hz, Ar-H), 7.33 (s, 2H, -), 7.35-7.38 (m, 2H, Ar-H), 7.46-7.51 (m, 3H, Ar-H), 7.91 (d, 1H, J = 7.65 Hz, Ar-H), 8.11 (d, 1H, J = 6.75 Hz, Ar-H), 11.98 (br s, 1H, -NH,)

SU8	δ 2.28 (s, 3H, -CH3), 4.34 (s , 1H, -CH), 7.13 (t, 1H, J = 7.65 Hz, Ar-H), 7.19-7.21 (m, 5H, Ar-H), 7.23 (s, 2H,), 7.48 (d, 1H, J = 7.65 Hz, Ar-H), 7.92 (d, 1H, J = 8.4 Hz, Ar-H), 8.09 (d, 1H, J = 6.73 Hz, Ar-H), 11.95 (br s, 1H, -NH,)
SU9	δ 2.38 (s, 3H, -CH3), 4.71 (s, 1H, -CH), 7.13 (t, 1H, J = 7.65 Hz, Ar-H), 7.18 (s, 2H, -), 7.19-7.22 (m, 5H, Ar-H), 7.48 (d, 1H, J = 7.65 Hz, Ar-H), 7.91 (d, 1H, J = 7.65 Hz, Ar-H), 8.08 (d, 1H, J = 6.7 Hz, Ar-H), 11.95 (br s, 1H, -NH,)
SU10	δ 2.9-3.04 (m, 6H, -CH3), 4.50 (s, 1H, -CH), 7.13 (t, 1H, J = 7.65 Hz, Ar-H), 7.19-7.21 (m, 5H, Ar-H), 7.23 (s, 2H, -NH) .48 (d, 1H, J = 7.65 Hz, Ar-H), 7.92 (d, 1H, J = 8.4 Hz, Ar-H), 8.10 (d, 1H, J = 6.73 Hz, Ar-H), 11.97 (br s, 1H, -NH,)
SU11	δ 3.69 (s, 3H, -OCH_3), 3.77 (s, 3H, -OCH_3), 4.79 (s, 1H, -CH), 7.13-7.14 (m, 1H, Ar-H), 7.18-7.20 (m, 3H, Ar-H), 7.25 (s, 2H, -NH_2,), 7.47 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.89 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.09 (d, 1H, *J* = 6.8 Hz, Ar-H), 11.95 (br s, 1H, -NH,)
SU12	δ 5.38 (s, 1H, -CH), 7.15 (t , 1H, *J* = 6.85 Hz, Ar-H), 7.22 (t, 1H, *J* = 7.65 Hz, Ar-H),), 7.48-7.56 (m, 5H, Ar-H), 7.89-7.98 (m, 3H, Ar-H), 8.10 (d, 1H, *J* = 7.25 Hz, Ar-H), 8.38 (d, 1H, *J* = 7.65 Hz, Ar-H), 11.97 (br s, 1H, -NH,)

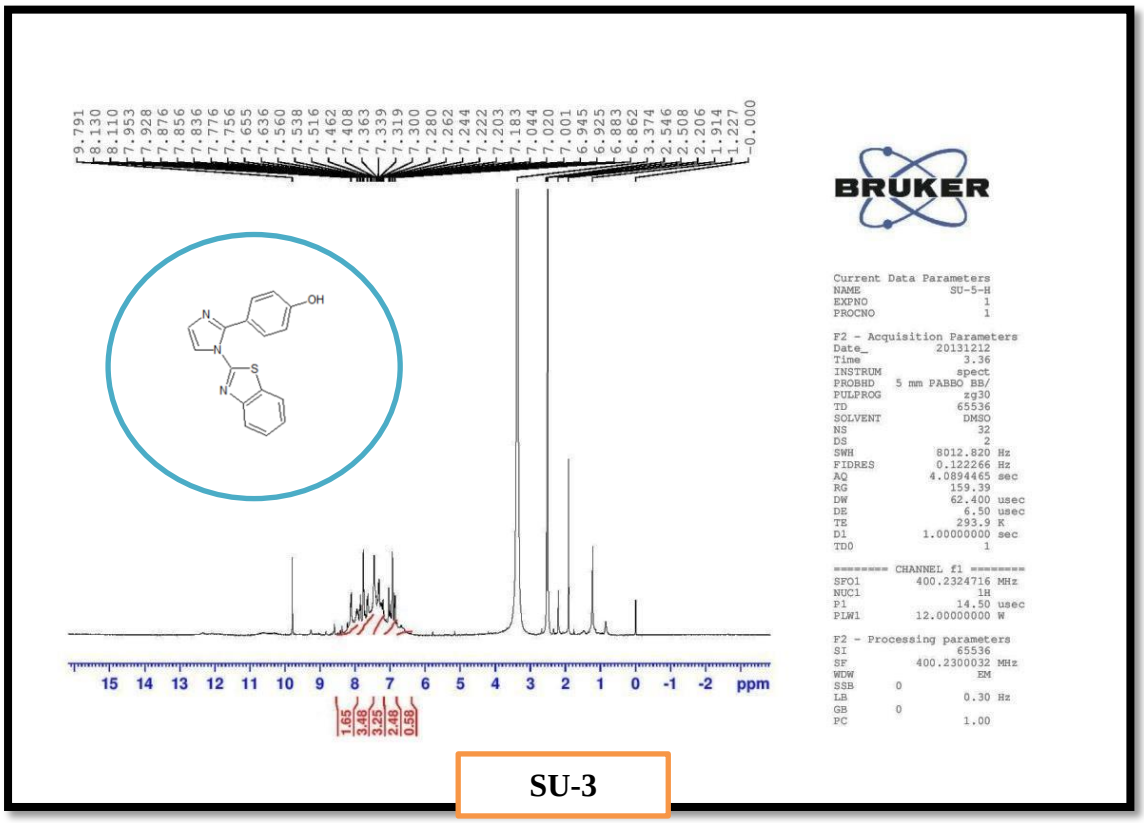
[1]H NMR Spectrum of the Synthesized Compounds



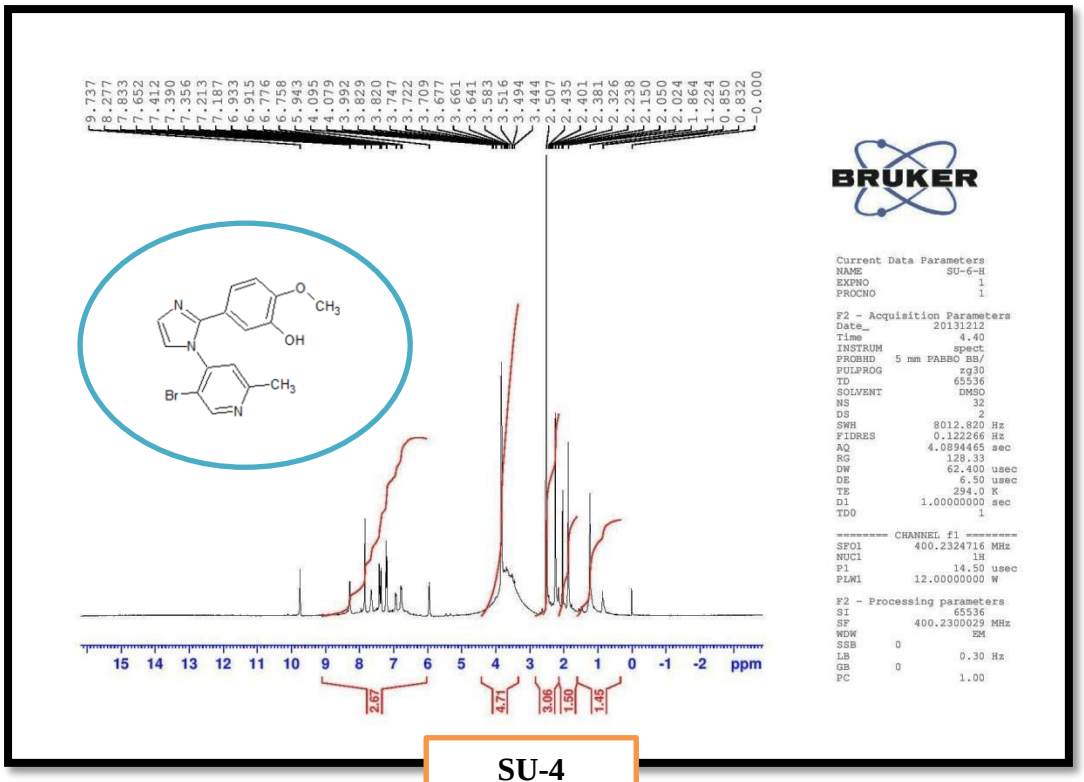
SU-1


BRUKER
Current Data Parameters
NAME SU-4R-H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 19.13
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 49.2
DW 62.400 usec
DE 6.50 usec
TE 293.3 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
1.88
2.80
3.83
3.49
3.49
0.75

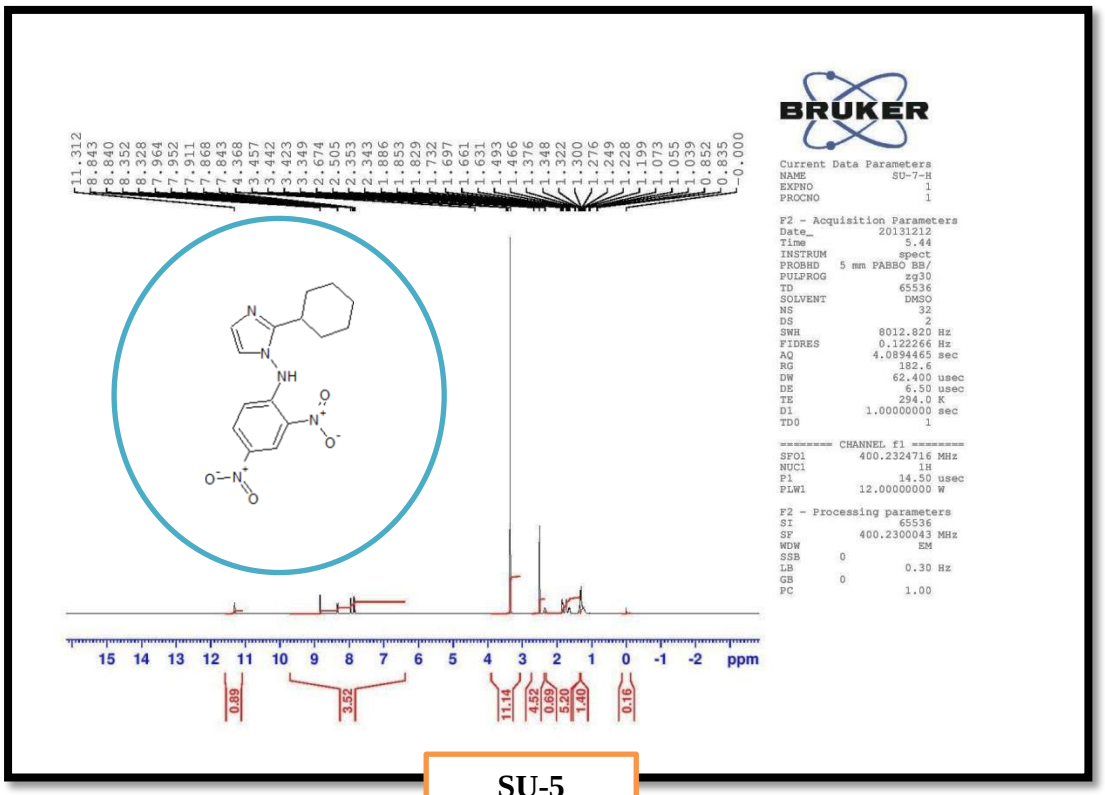
SU-2


9.791
8.130
8.110
7.953
7.928
7.876
7.856
7.836
7.776
7.756
7.655
7.636
7.560
7.538
7.516
7.462
7.408
7.363
7.339
7.319
7.300
7.280
7.262
7.244
7.222
7.203
7.183
7.044
7.020
7.001
6.945
6.925
6.883
6.862
3.374
2.546
2.508
2.206
1.914
1.227
0.000
BRUKER
Current Data Parameters
NAME SU-5-H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 3.36
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 159.39
DW 62.400 usec
DE 6.50 usec
TE 293.9 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
1.65
3.48
3.25
2.48
0.58

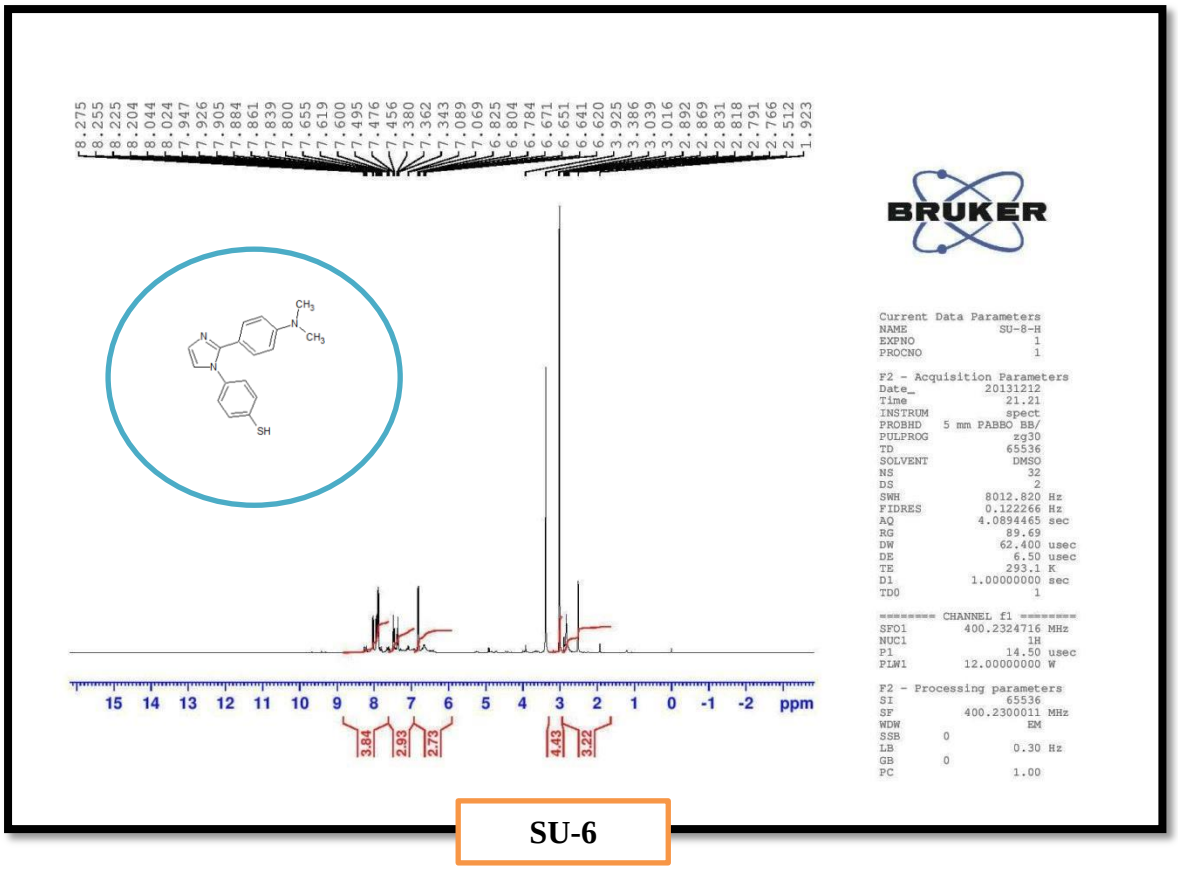
SU-3


BRUKER
Current Data Parameters
NAME SU-6-H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 4.40
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 128.33
DW 62.400 usec
DE 6.50 usec
TE 294.0 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300029 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm

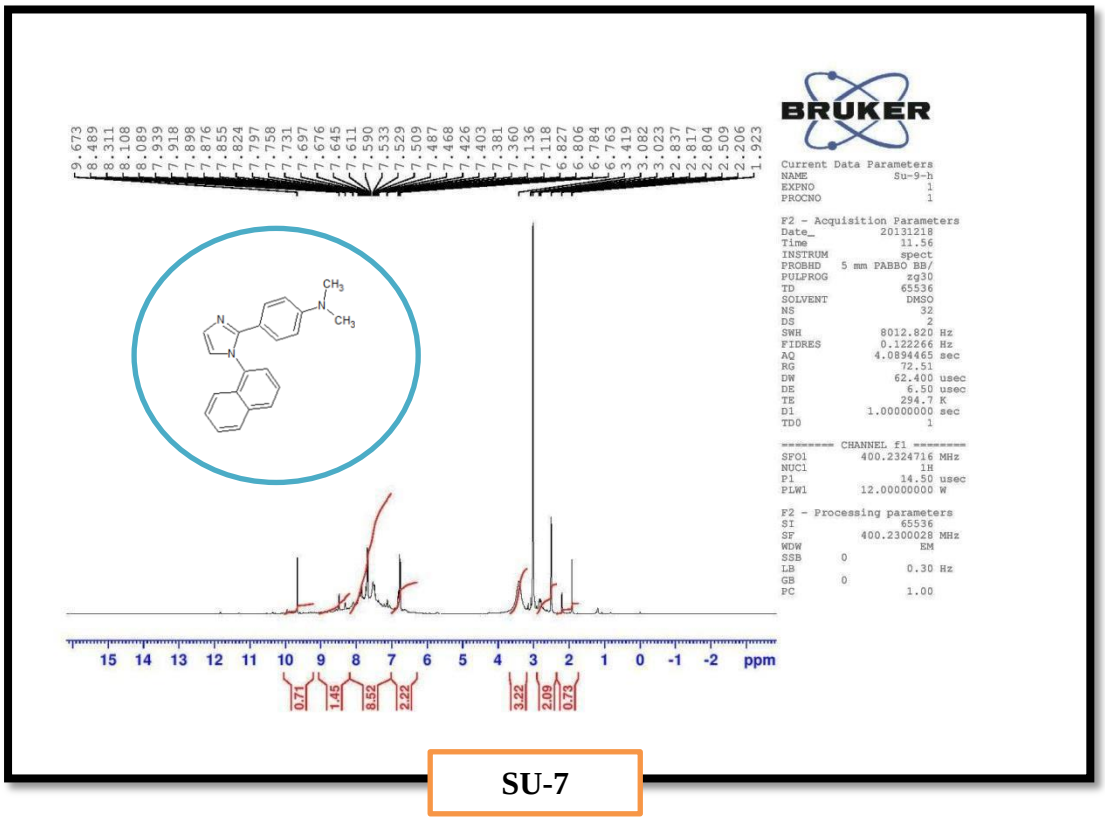
SU-4


BRUKER
Current Data Parameters
NAME SU-7-H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 5.44
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 182.6
DW 62.400 usec
DE 6.50 usec
TE 294.0 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300043 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm

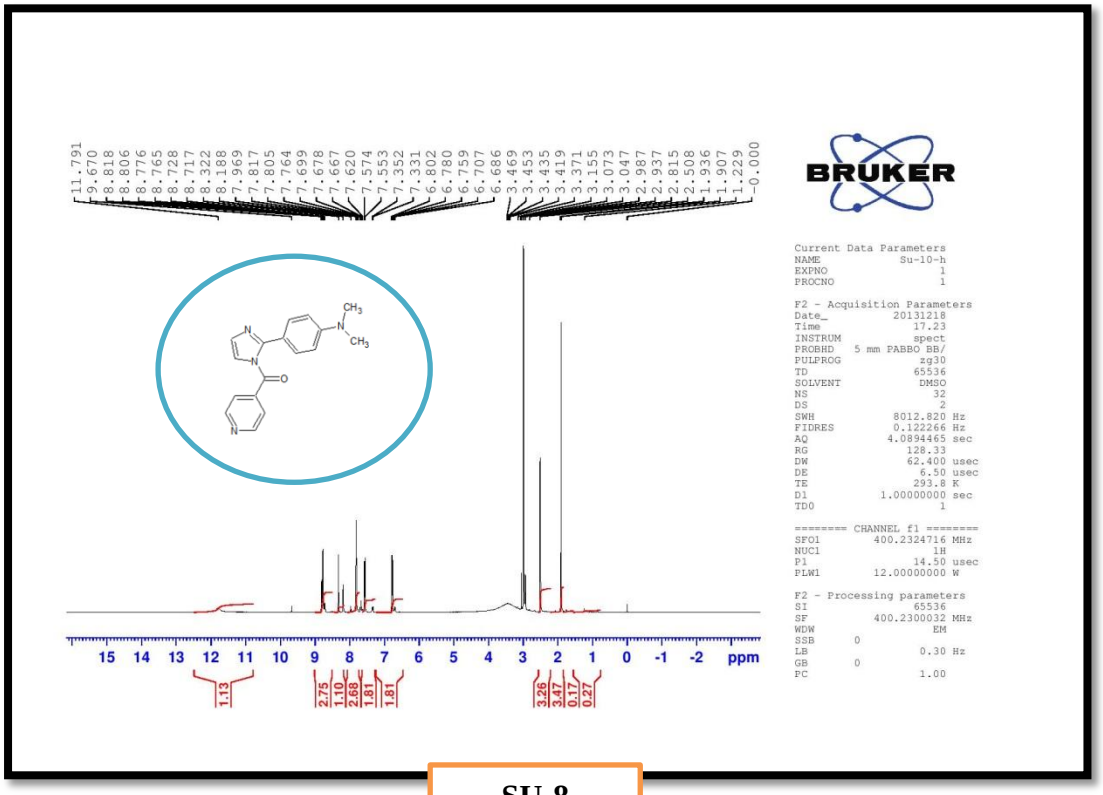
SU-5


8.275 8.255 8.225 8.204 8.044 8.024 7.947 7.926 7.905 7.884 7.861 7.839 7.800 7.655 7.619 7.600 7.495 7.476 7.456 7.380 7.362 7.343 7.089 7.069 6.825 6.804 6.784 6.671 6.651 6.641 6.620 3.925 3.386 3.039 3.016 2.892 2.869 2.831 2.818 2.791 2.766 2.512 1.923
BRUKER
Current Data Parameters
NAME SU-8-H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 21.21
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 89.69
DW 62.400 usec
DE 6.50 usec
TE 293.1 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300011 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
3.84 2.93 2.73 4.43 3.22

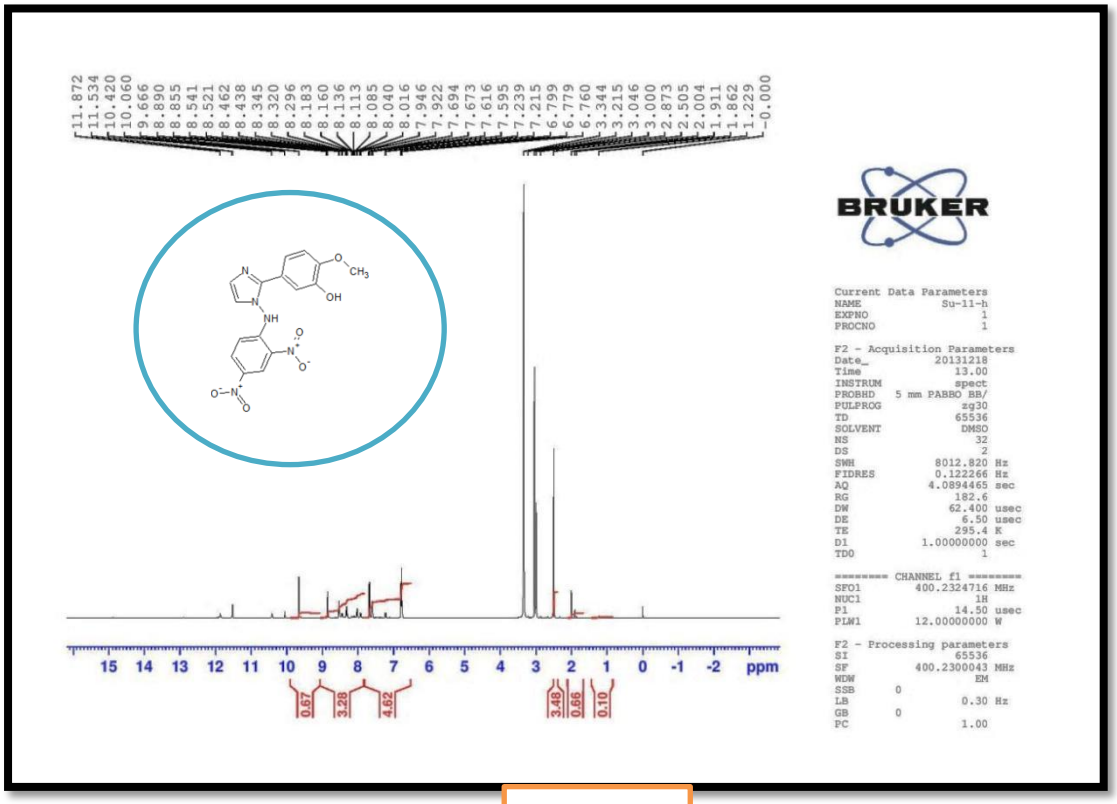
SU-6


9.673 8.489 8.311 8.108 8.089 7.939 7.918 7.898 7.876 7.855 7.824 7.797 7.758 7.731 7.697 7.676 7.645 7.611 7.590 7.533 7.529 7.509 7.487 7.468 7.426 7.403 7.381 7.360 7.136 7.118 6.827 6.806 6.784 6.763 3.419 3.082 3.023 2.837 2.817 2.804 2.509 2.206 1.923
BRUKER
Current Data Parameters
NAME Su-9-h
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 11.56
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 72.51
DW 62.400 usec
DE 6.50 usec
TE 294.7 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300028 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
0.71 1.45 8.52 2.22 3.22 2.09 0.73

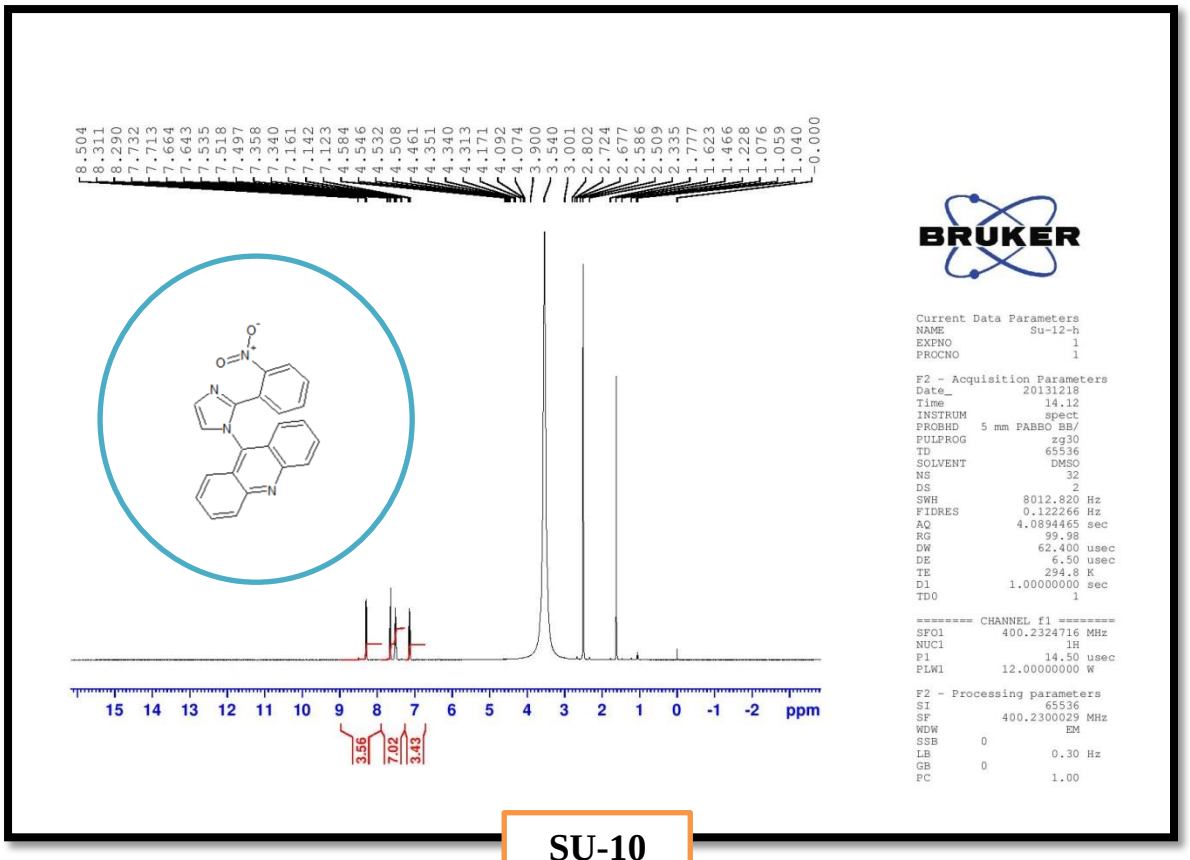
SU-7


11.791
9.670
8.818
8.806
8.776
8.765
8.728
8.717
8.322
8.188
7.969
7.817
7.805
7.764
7.699
7.678
7.667
7.620
7.574
7.553
7.352
7.331
6.802
6.780
6.759
6.707
6.686
3.469
3.453
3.435
3.419
3.371
3.155
3.073
3.047
2.987
2.937
2.815
2.508
1.936
1.907
1.229
0.000
BRUKER
Current Data Parameters
NAME Su-10-h
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 17.23
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 128.33
DW 62.400 usec
DE 6.50 usec
TE 293.8 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
1.13
2.75
1.10
2.68
1.81
1.81
3.26
3.47
0.17
0.27

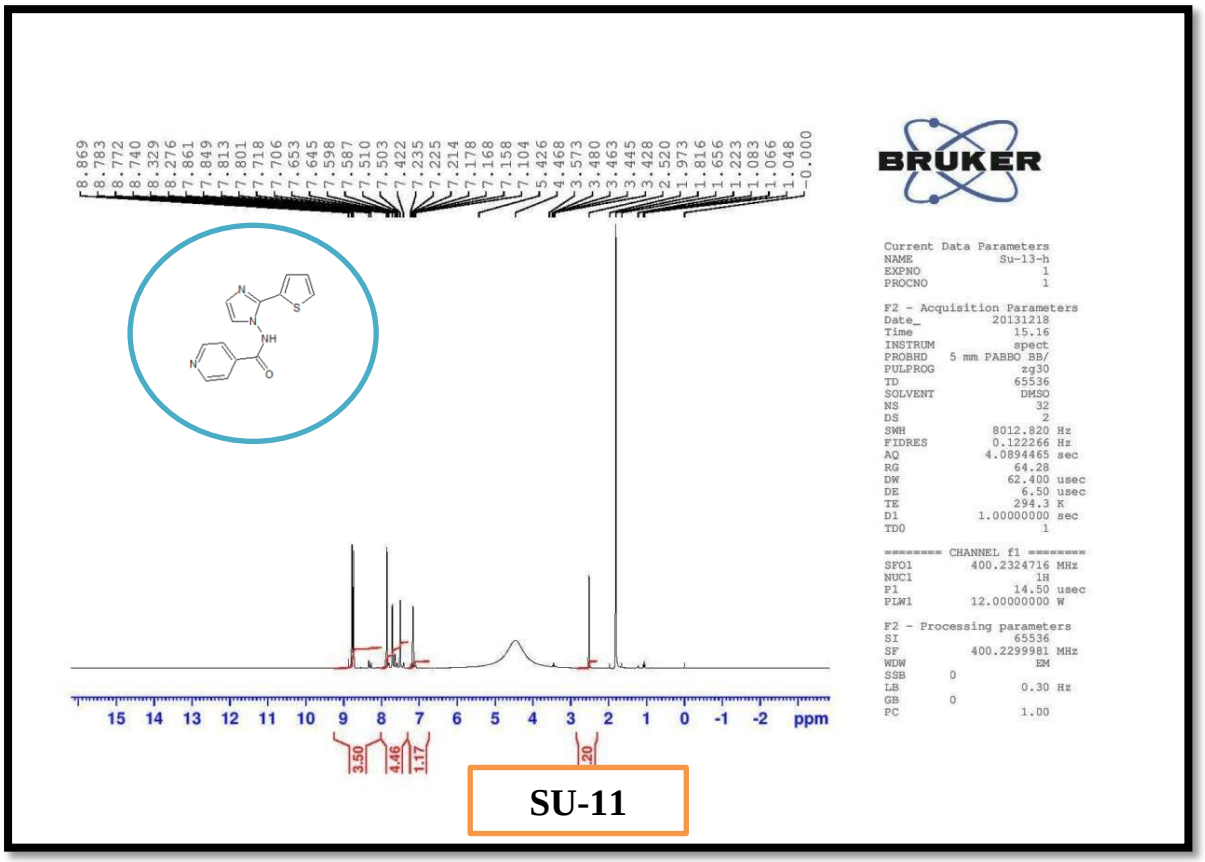
SU-8


11.872
11.534
10.420
10.060
9.666
8.890
8.855
8.541
8.521
8.462
8.438
8.345
8.320
8.296
8.183
8.160
8.136
8.113
8.085
8.040
8.016
7.946
7.922
7.694
7.673
7.616
7.595
7.239
7.215
6.799
6.779
6.760
3.344
3.215
3.046
3.000
2.873
2.505
2.004
1.911
1.862
1.229
0.000
BRUKER
Current Data Parameters
NAME Su-11-h
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 13.00
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 182.6
DW 62.400 usec
DE 6.50 usec
TE 295.4 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300043 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
0.67
3.28
4.62
3.48
0.86
0.10

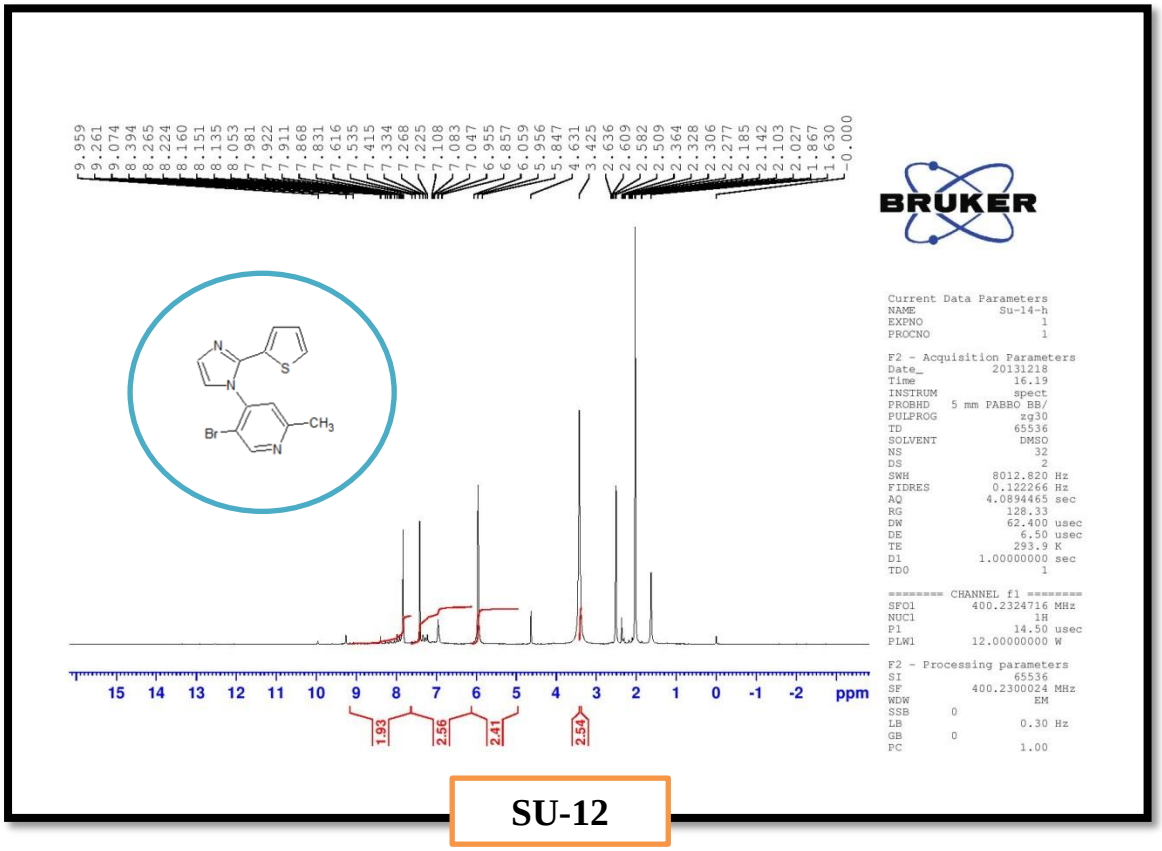
SU-9


8.504
8.311
8.290
7.732
7.713
7.664
7.643
7.535
7.518
7.497
7.358
7.340
7.161
7.142
7.123
4.584
4.546
4.532
4.508
4.461
4.351
4.340
4.313
4.171
4.092
4.074
3.900
3.540
3.001
2.802
2.724
2.677
2.586
2.509
2.335
1.777
1.623
1.466
1.228
1.076
1.059
1.040
0.000
BRUKER
Current Data Parameters
NAME Su-12-h
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 14.12
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 99.98
DW 62.400 usec
DE 6.50 usec
TE 294.8 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300029 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
3.56
7.02
3.43

SU-10


8.869
8.783
8.772
8.740
8.329
8.276
7.861
7.849
7.813
7.801
7.718
7.706
7.653
7.645
7.598
7.587
7.510
7.503
7.422
7.235
7.225
7.214
7.178
7.168
7.158
7.104
5.426
4.468
3.573
3.480
3.463
3.445
3.428
2.520
1.973
1.816
1.656
1.223
1.083
1.066
1.048
0.000
BRUKER
Current Data Parameters
NAME Su-13-h
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 15.16
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 64.28
DW 62.400 usec
DE 6.50 usec
TE 294.3 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2299981 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
3.50
4.46
1.17
20

SU-11


9.959
9.261
9.074
8.394
8.265
8.224
8.160
8.151
8.135
8.053
7.981
7.922
7.911
7.868
7.831
7.616
7.535
7.415
7.334
7.268
7.225
7.108
7.083
7.047
6.955
6.857
6.059
5.956
5.847
4.631
3.425
2.636
2.609
2.582
2.509
2.364
2.328
2.306
2.277
2.185
2.142
2.103
2.027
1.867
1.630
0.000
BRUKER
Current Data Parameters
NAME Su-14-h
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 16.19
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 128.33
DW 62.400 usec
DE 6.50 usec
TE 293.9 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.2324716 MHz
NUC1 1H
P1 14.50 usec
PLW1 12.00000000 W
F2 - Processing parameters
SI 65536
SF 400.2300024 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
N
S
N
Br
CH3
N
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
1.93
2.56
2.41
2.54

SU-12

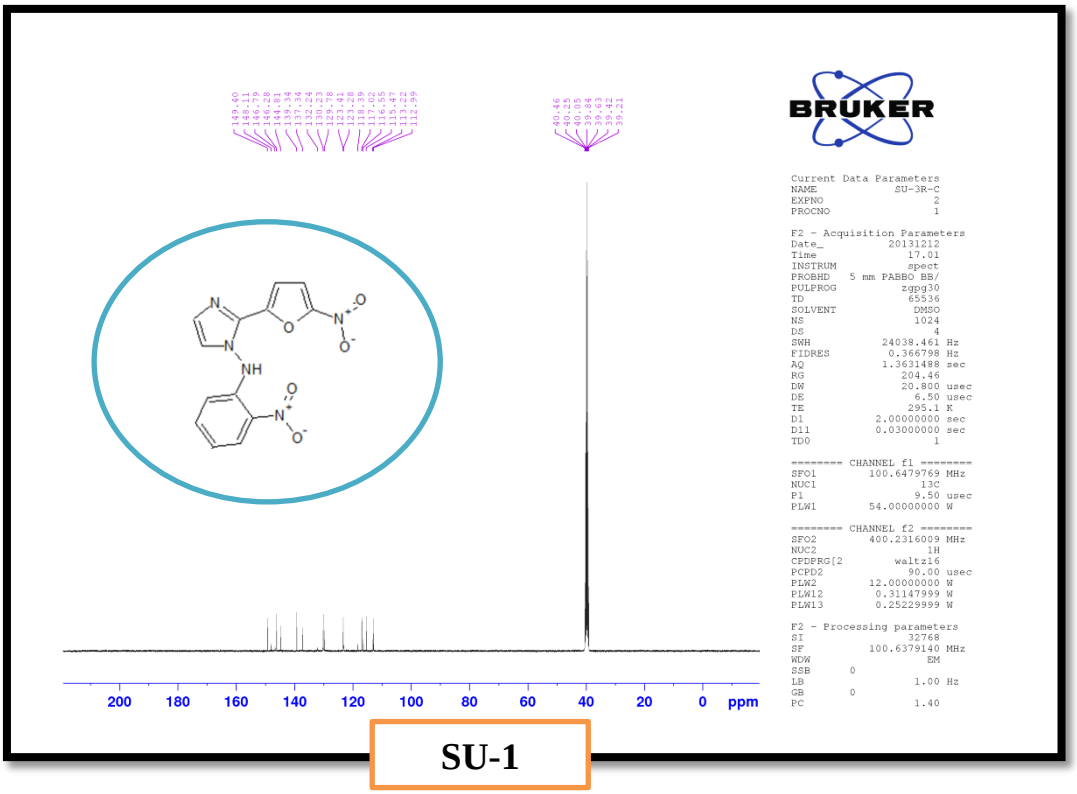
A) ^{13}C NMR Spectral Data of the Synthesized Compounds-

Table-10

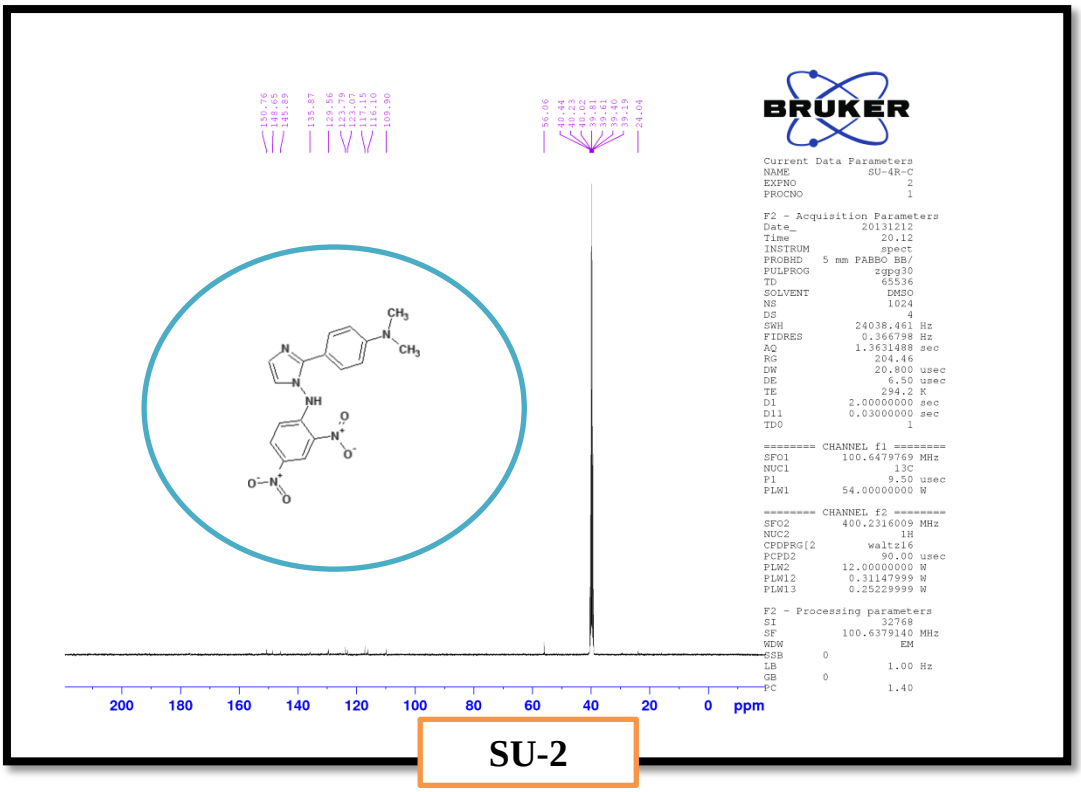
Compounds	**^{13}C NMR Data** (500 MHz, DMSO-d_6)
SU1	δ 19.09, 39.85, 40.01, 40.18, 56.44, 56.59, 84.62, 105.87, 112.82, 121.48, 122.04, 123.18, 128.13, 128.24, 129.46, 129.83, 136.46, 143.59, 156.23, 159.20.
SU2	δ 31.21, 55.99, 84.10, 105.80, 112.83, 119.33, 119.64, 121.51, 122.06, 123.21, 125.00, 129.46, 129.94, 130.12, 132.86, 136.46, 142.5, 156.40, 159.23.
SU3	δ 31.21, 55.93, 84.04, 105.79, 112.84, 119.32, 119.63, 121.44, 121.52, 122.05, 123.22, 124.98, 129.95, 130.47, 132.39, 136.46, 142.98, 156.40, 159.22.
SU4	δ 31.20, 56.33, 84.49, 105.84, 112.82, 116.15, 116.32, 119.37, 119.69, 121.49, 122.03, 123.20. 125.00, 129.86, 130.16, 130.23, 136.46, 139.81.
SU5	δ 31.12, 54.96, 82.94, 105.78, 112.84, 119.14, 119.50, 121.54, 121.99, 123.24, 124.99, 128.66, 129.90, 130.18, 130.65, 131.53, 133.01, 136.44, 139.79.
SU6	δ 19.09, 54.88, 56.58, 82.92, 105.81, 112.84, 116.43, 116.60, 119.29, 119.62, 121.51, 122.00, 123.20, 124.99, 125.61, 129.91, 130.47, 130.66, 136.47.

SU7	δ 31.21, 55.78, 83.84, 105.74, 112.85, 119.30, 119.62, 121.57, 122.02, 122.61, 123.26, 124.95, 127.39, 130.02, 130.85, 131.25, 131.80, 136.44, 146.26, 156.53, 159.29.
SU8	δ 31.20, 55.37, 83.38, 105.68, 112.87, 119.21, 119.53, 121.62, 122.04, 122.68, 123.30, 123.45, 124.94, 130.17, 131.29, 135.10, 136.46, 145.75, 148.62, 156.86, 159.45.
SU9	δ 21.23, 39.84, 40.13, 40.17, 56.57, 84.84, 105.91, 112.81, 119.48, 121.46, 122.03, 123.17, 125.01, 128.05, 129.75, 130.01, 136.45, 140.67, 156.03, 159.11.
SU10	δ 31.20, 55.24, 56.31, 56.42, 83.39, 105.88, 112.82, 113.43, 119.10, 119.50, 121.48, 121.95, 123.20, 125.02, 129.85, 136.43, 149.24, 149.64, 156.64, 159.40.
SU11	δ 31.22, 55.22, 83.15, 102.86, 105.85, 112.79, 112.95, 113.71, 119.07, 119.42, 121.45, 122.16, 123.17, 125.03, 129.93, 136.43, 148.51, 156.75, 159.39.
SU12	δ 39.85, 40.02, 40.18, 56.80, 84.95, 105.89, 112.85, 119.41, 119.75, 121.51, 122.06, 123.20, 123.62, 125.06, 126.48, 126.87, 128.85, 129.36, 129.84, 131.47, 134.19, 136.48.

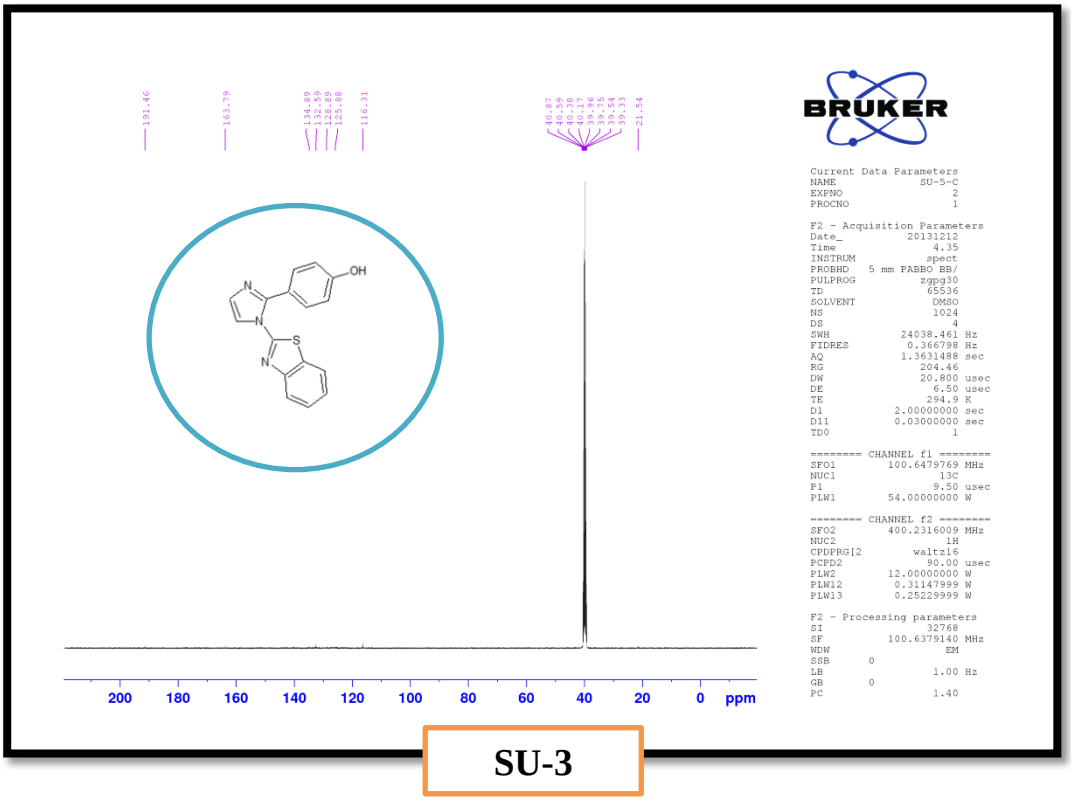
^{13}C NMR Spectrum of the Synthesized Compounds



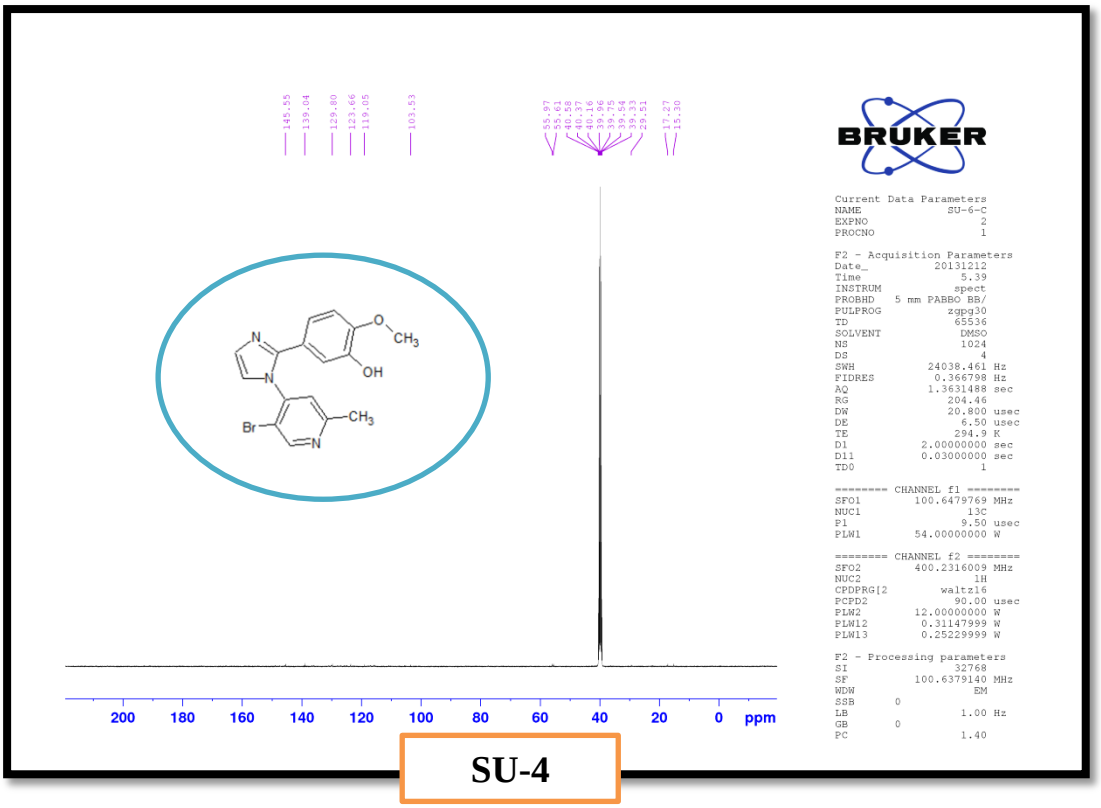
SU-1


150.76
148.65
145.89
138.87
129.56
123.09
121.07
117.15
116.10
109.90
56.06
40.44
40.23
40.02
39.81
39.61
39.40
39.19
24.04
BRUKER
Current Data Parameters
NAME SU-4R-C
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 20.12
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 294.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

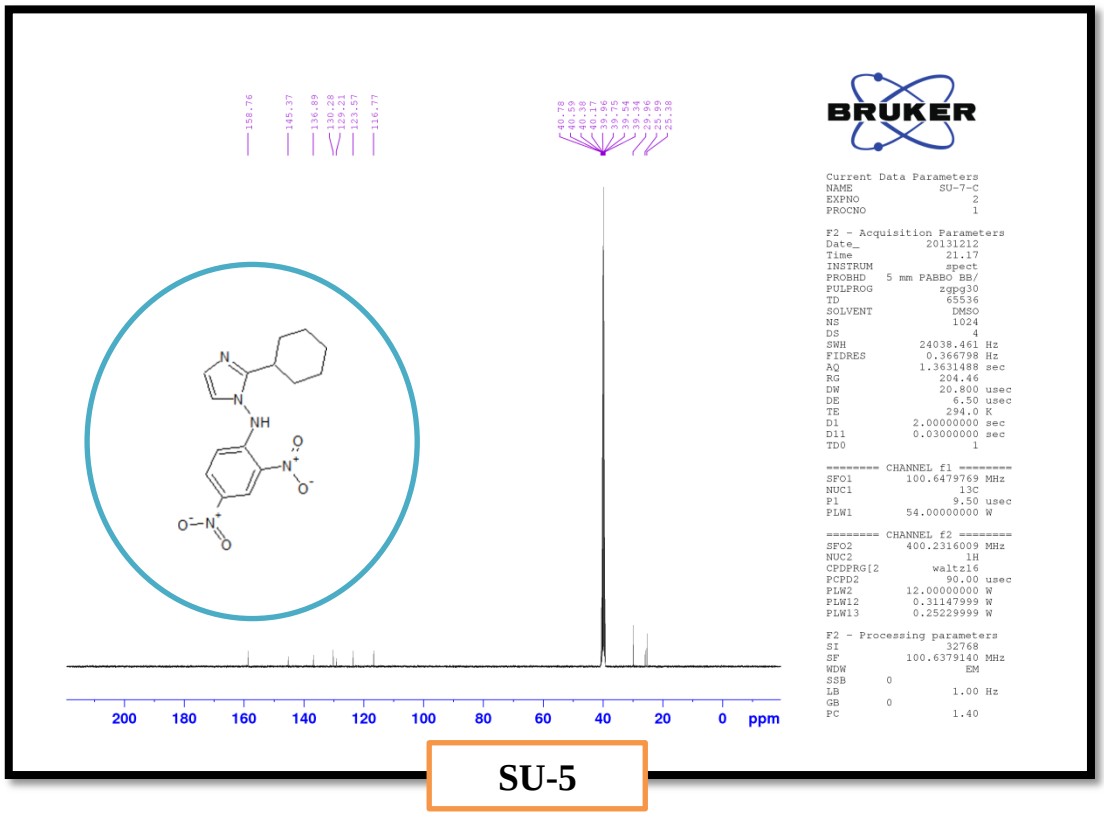
SU-2


191.46
163.79
134.89
132.59
128.89
125.88
116.31
40.67
40.59
40.38
40.17
39.96
39.75
39.54
39.33
21.54
BRUKER
Current Data Parameters
NAME SU-5-C
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 4.35
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 294.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

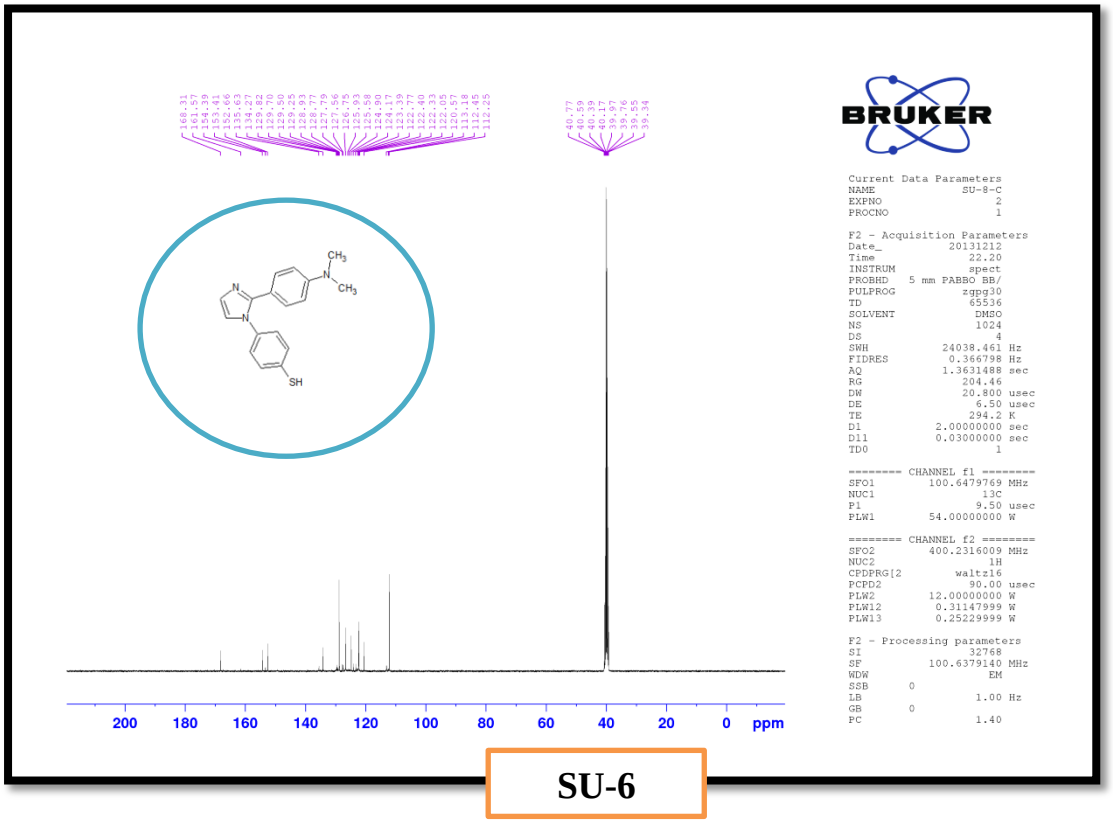
SU-3


145.55
139.04
129.80
123.66
119.05
103.53
55.97
55.61
40.58
40.37
40.16
39.96
39.75
39.54
39.33
29.51
17.27
15.30
BRUKER
Current Data Parameters
NAME SU-6-C
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 5.39
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 294.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

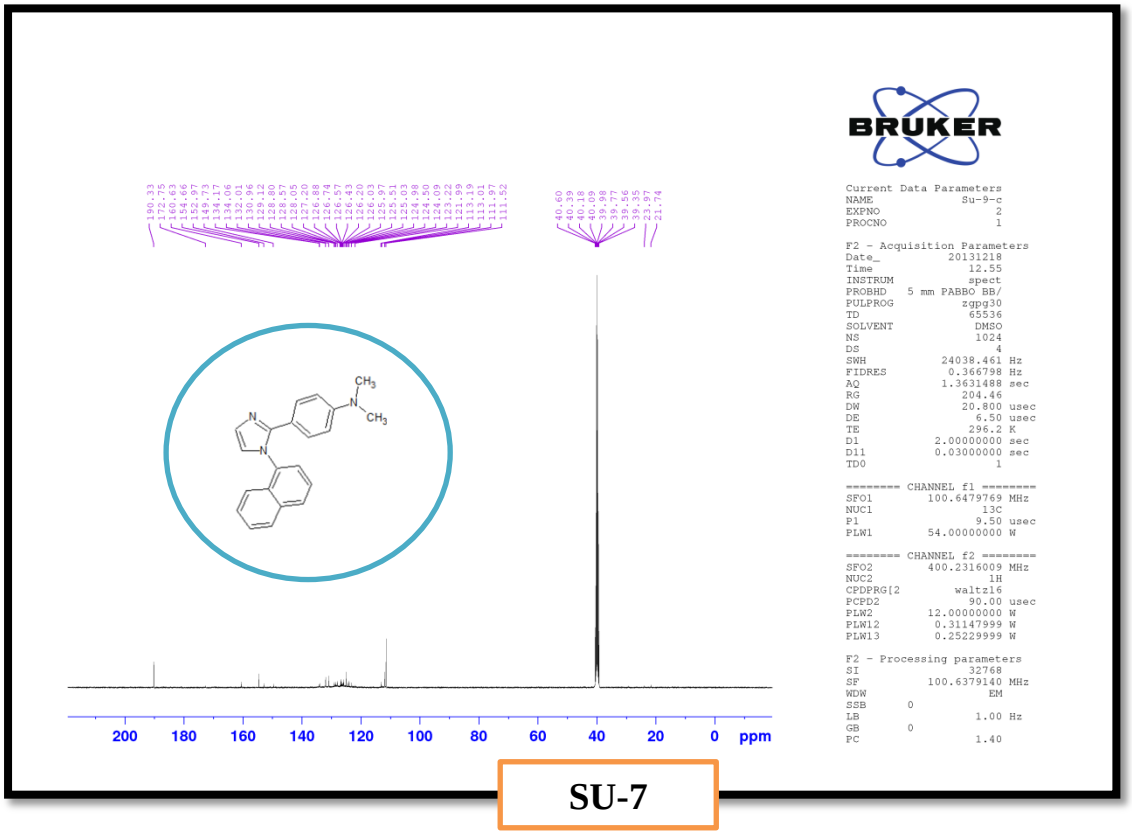
SU-4


158.76
145.37
136.89
130.28
129.21
123.57
116.77
40.78
40.58
40.38
40.17
39.96
39.75
39.54
39.34
29.96
25.99
25.38
BRUKER
Current Data Parameters
NAME SU-7-C
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 21.17
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 294.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

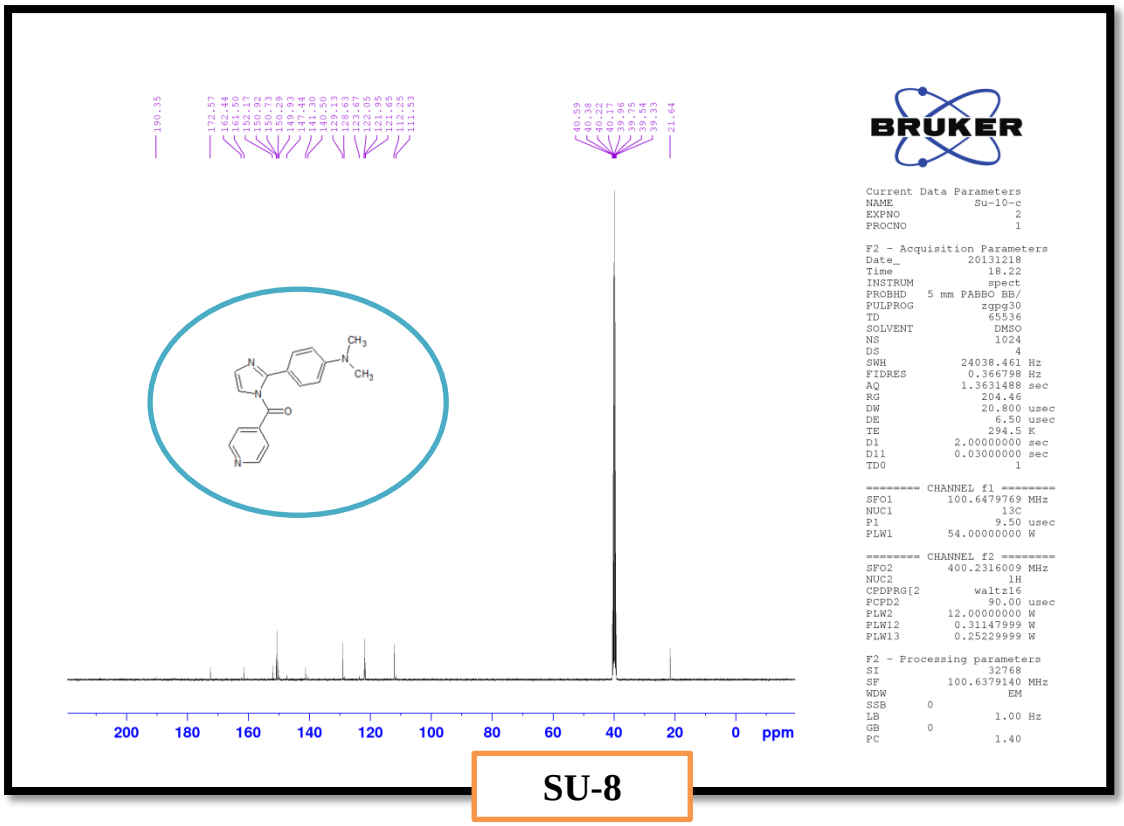
SU-5


BRUKER
Current Data Parameters
NAME SU-8-c
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131212
Time 22.20
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 294.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

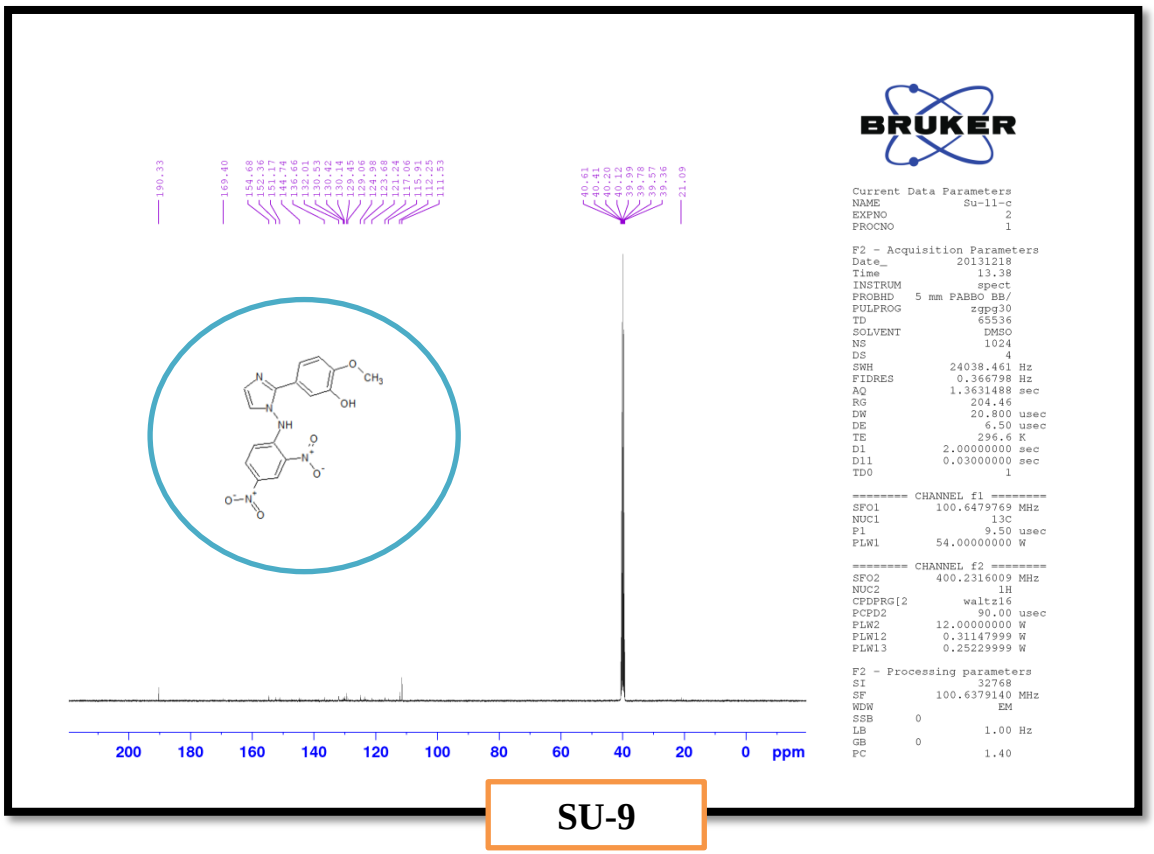
SU-6


BRUKER
Current Data Parameters
NAME Su-9-c
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 12.55
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 296.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

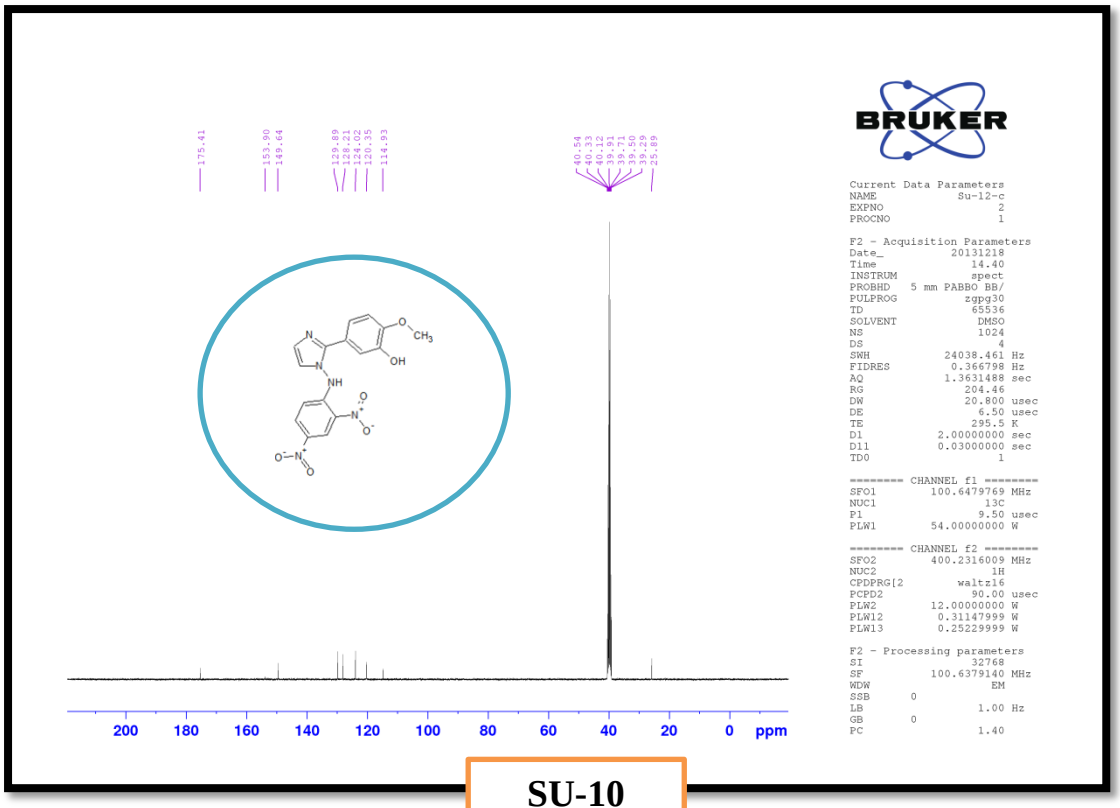
SU-7


190.35
172.57
162.44
161.50
152.17
150.92
150.73
150.29
149.93
147.44
141.30
140.50
139.13
128.63
123.67
122.05
121.65
121.60
112.25
111.53
40.59
40.38
40.22
40.17
39.96
39.75
39.54
39.33
21.64
200 180 160 140 120 100 80 60 40 20 0 ppm
BRUKER
Current Data Parameters
NAME Su-10-c
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 18.22
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 294.5 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

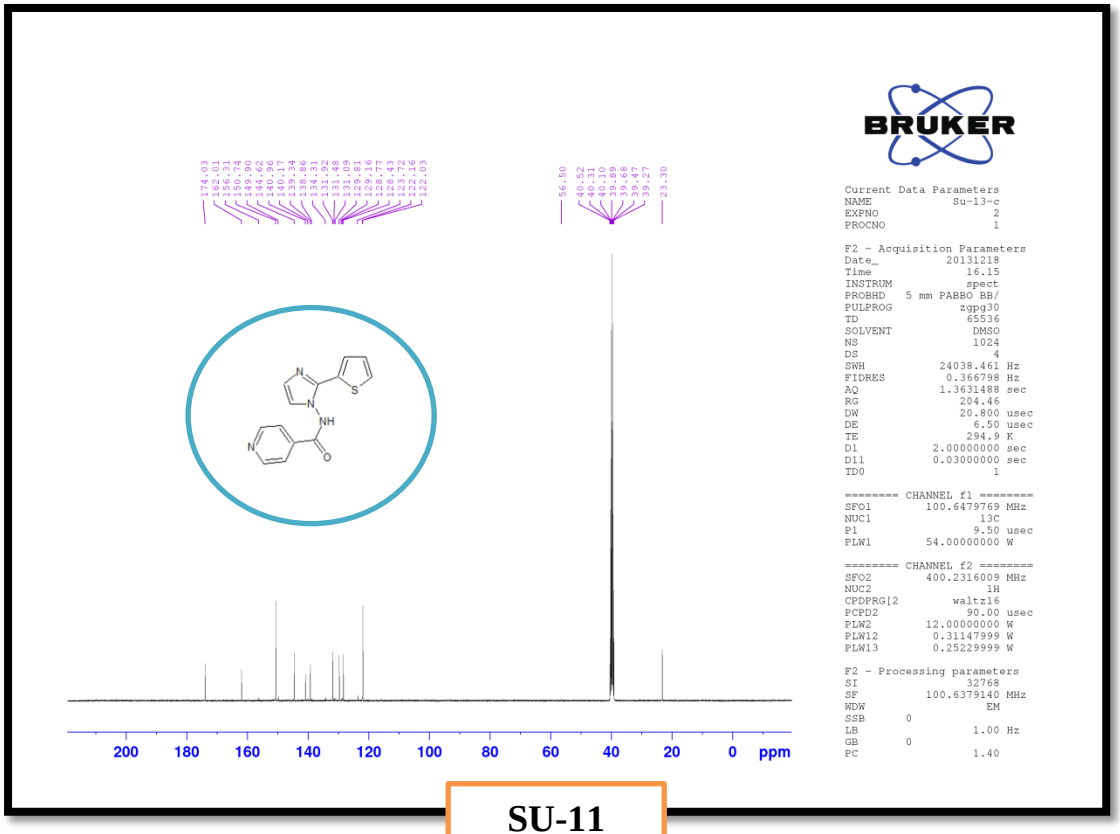
SU-8


190.33
169.40
154.68
152.36
151.17
144.74
144.66
132.61
130.53
130.42
130.14
130.05
129.06
124.98
123.68
121.24
121.06
115.91
112.25
111.53
40.61
40.41
40.20
40.12
40.00
39.78
39.57
39.36
21.09
200 180 160 140 120 100 80 60 40 20 0 ppm
BRUKER
Current Data Parameters
NAME Su-11-c
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 13.38
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 296.6 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

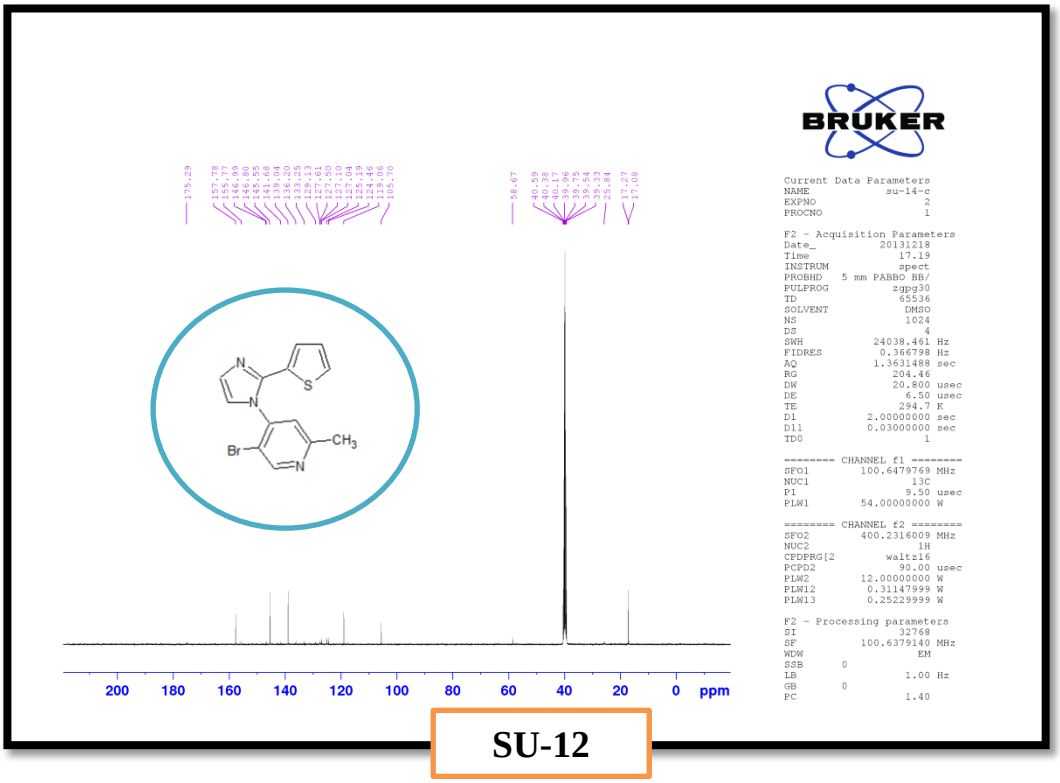
SU-9


175.41
153.90
149.64
129.89
128.21
124.02
120.35
114.93
40.54
40.33
40.12
39.91
39.71
39.50
39.29
25.89
BRUKER
Current Data Parameters
NAME Su-12-c
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 14.40
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 295.5 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

SU-10


174.03
162.01
156.31
150.78
149.90
144.62
140.96
140.17
139.14
138.86
134.31
131.92
131.68
131.09
129.81
129.16
128.77
128.43
123.72
122.16
122.03
56.50
40.52
40.31
40.10
39.89
39.68
39.47
39.27
23.30
BRUKER
Current Data Parameters
NAME Su-13-c
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 16.15
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 294.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

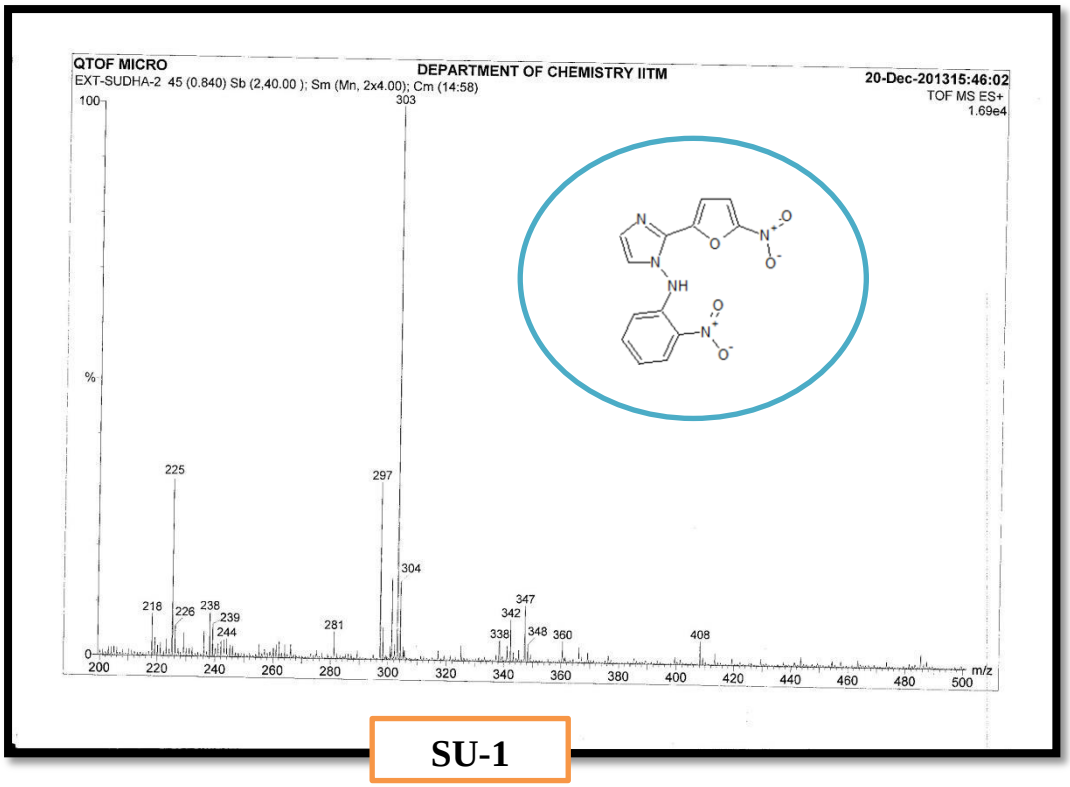
SU-11


BRUKER
Current Data Parameters
NAME su-14-c
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131218
Time 17.19
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1024
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 204.46
DW 20.800 usec
DE 6.50 usec
TE 294.7 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6479769 MHz
NUC1 13C
P1 9.50 usec
PLW1 54.00000000 W
======== CHANNEL f2 ========
SFO2 400.2316009 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 12.00000000 W
PLW12 0.31147999 W
PLW13 0.25229999 W
F2 - Processing parameters
SI 32768
SF 100.6379140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
175.29
157.78
157.77
146.99
146.80
141.58
141.58
139.04
136.20
129.23
127.61
127.50
127.30
127.04
126.19
124.46
121.06
105.70
58.67
40.59
40.38
40.17
39.96
39.75
39.54
39.33
25.84
17.27
17.08
N
S
N
Br
CH3
N
200
180
160
140
120
100
80
60
40
20
0
ppm

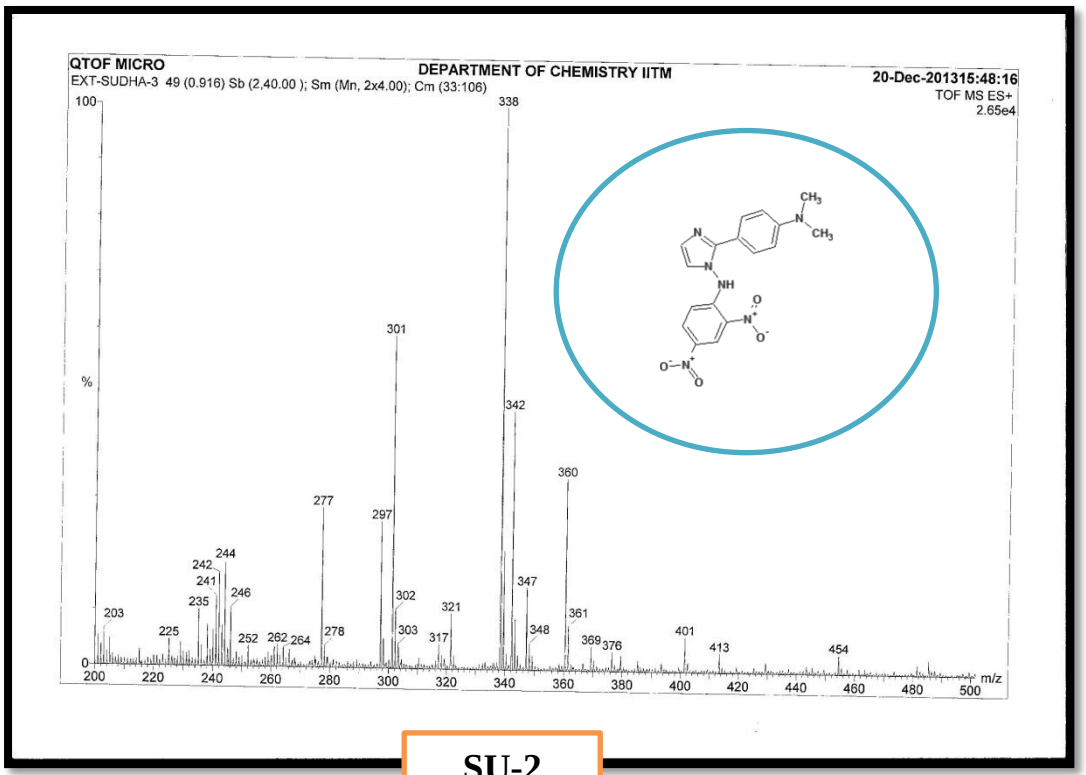
SU-12

A) **MASS spectral data of the synthesized compounds-** Table-11

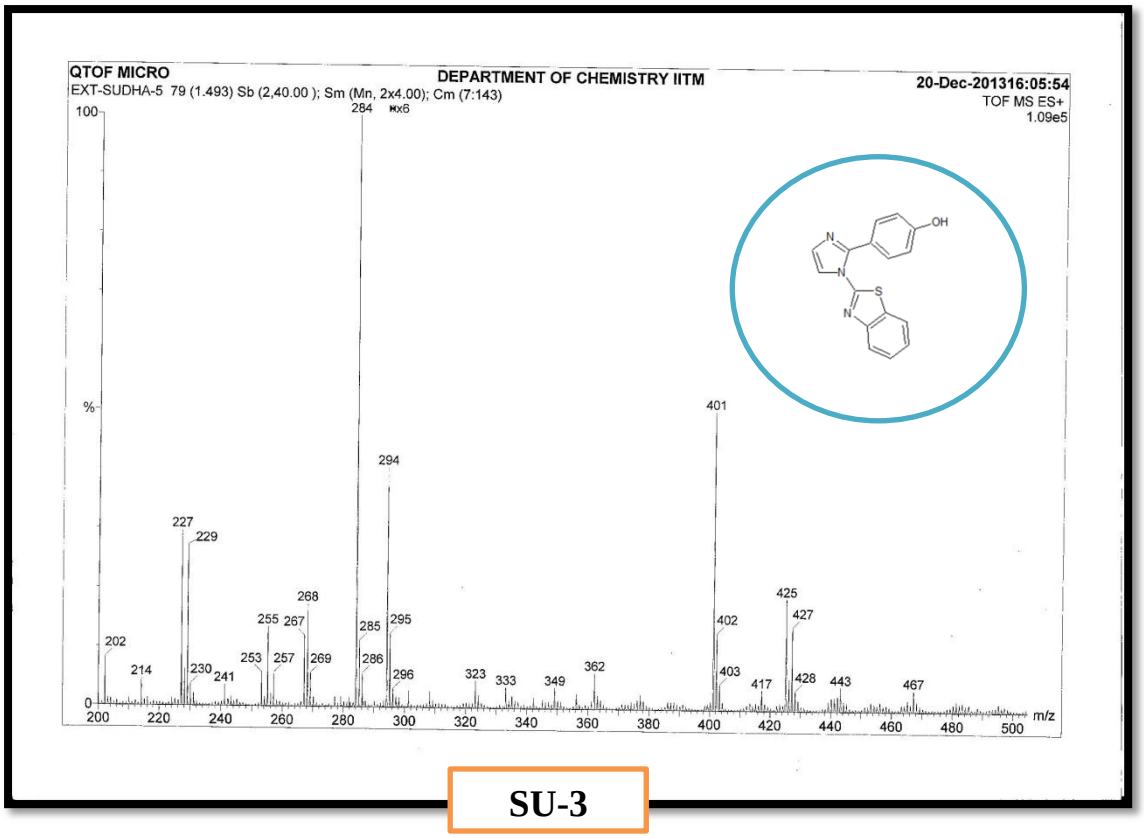
Compounds	Mass spectral data	Molecular weight
SU1	360(M^+)7%303(B)100%	360
SU2	369(M^+1)8%338(B)100%	369
SU3	294(M^+)45%295(M^+2)30%284(B)100%	293
SU4	360(M^+)12%361(M^+2)100%	360
SU5	331(M^+)5%485(B)100%	331
SU6	295(M^+)55%255(B)100%	295
SU7	313(M^+)50%275(B)100%	313
SU8	291(M^+)90%269(B)100%	291
SU9	371(M^+)25%261(B)100%	371
SU10	366(M^+)70%452(B)100%	366
SU11	273(M^+2)10%232(B)100%	270
SU12	320(M^+)100%322(M^+2)95%238(B)70%	320


QTOF MICRO
DEPARTMENT OF CHEMISTRY IITM
EXT-SUDHA-2 45 (0.840) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (14:58)
20-Dec-201315:46:02
TOF MS ES+
1.69e4
100
%
0
303
225
297
304
218
226
238
239
244
281
347
342
338
348
360
408
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z
NH

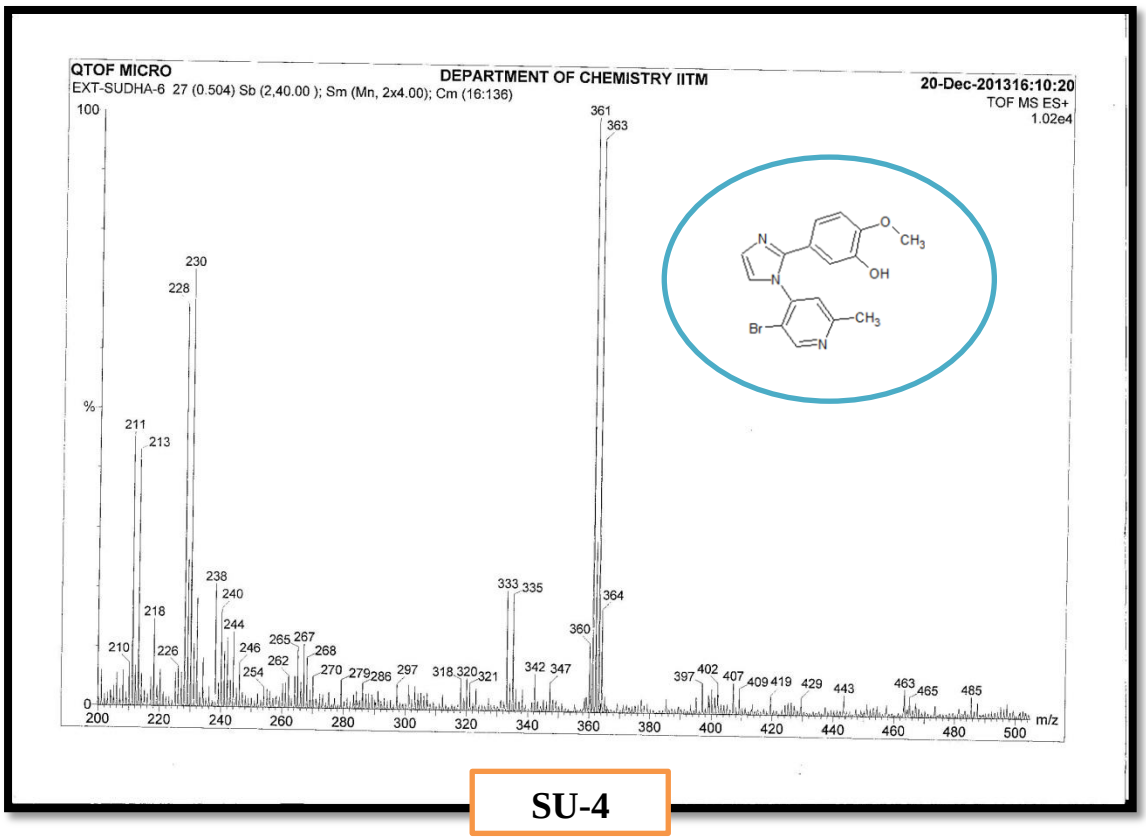
SU-1


QTOF MICRO
DEPARTMENT OF CHEMISTRY IITM
EXT-SUDHA-3 49 (0.916) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (33:106)
20-Dec-201315:48:16
TOF MS ES+
2.65e4
100
%
0
338
301
342
360
277
297
244
242
241
246
235
203
225
252
262
264
278
302
303
321
317
347
348
361
369
376
401
413
454
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z
NH
CH3
CH3

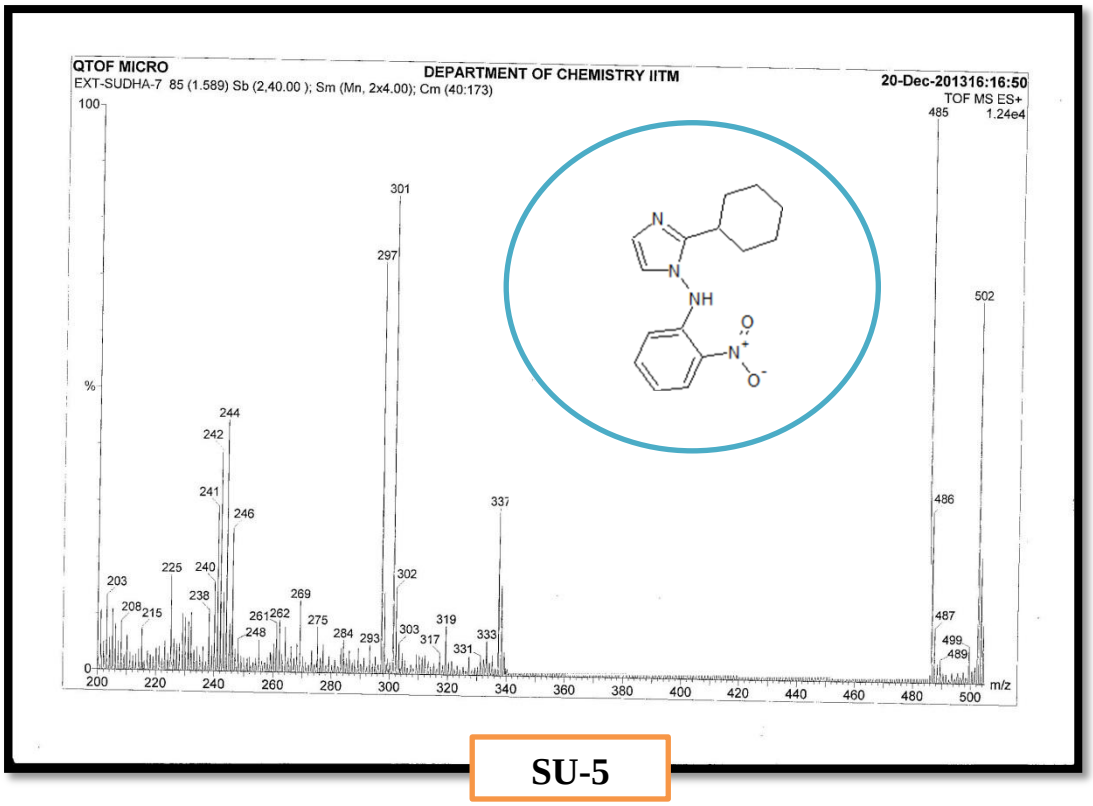
SU-2


QTOF MICRO
DEPARTMENT OF CHEMISTRY IITM
EXT-SUDHA-5 79 (1.493) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (7:143)
20-Dec-201316:05:54
TOF MS ES+
1.09e5
100
%
0
284
401
294
227
229
268
425
427
202
214
230
241
253
255
257
267
269
285
286
295
296
323
333
349
362
402
403
417
428
443
467
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z
OH
N
S

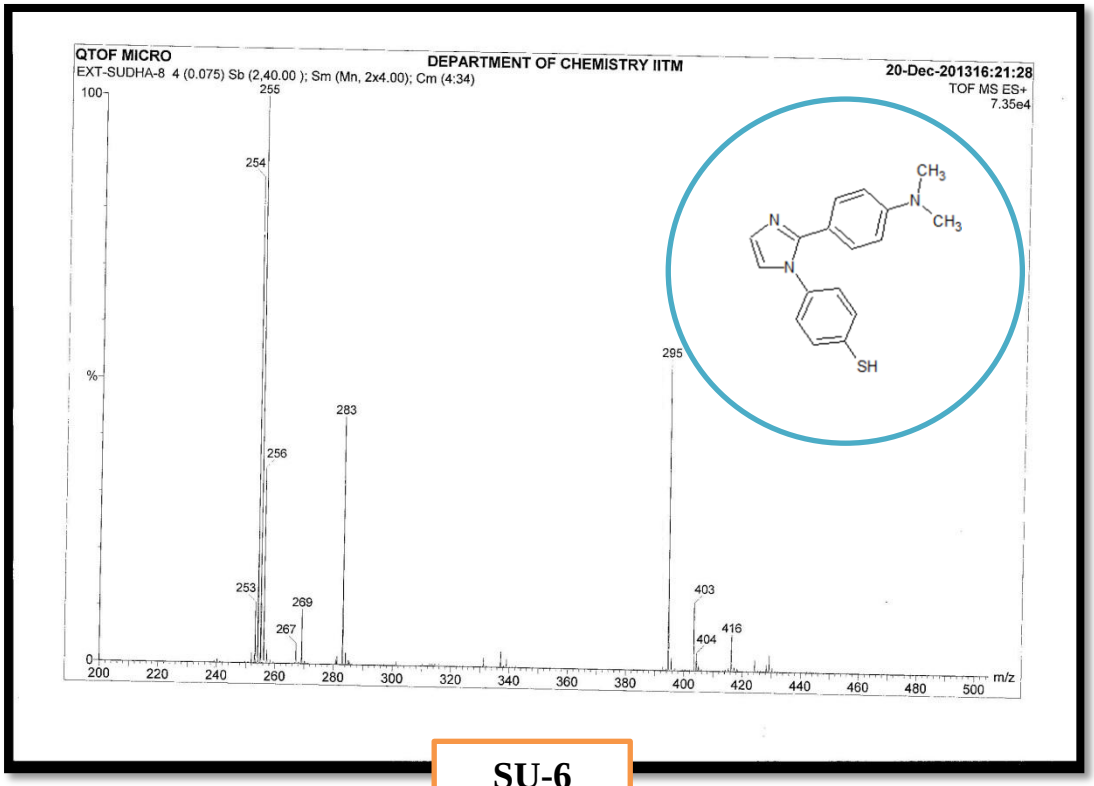
SU-3


QTOF MICRO
DEPARTMENT OF CHEMISTRY IITM
EXT-SUDHA-6 27 (0.504) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (16:136)
20-Dec-201316:10:20
TOF MS ES+
1.02e4
100
%
0
361
363
230
228
211
213
238
240
218
244
210
226
246
254
265
267
262
268
270
279
286
297
318
320
321
333
335
342
347
360
364
397
402
407
409
419
429
443
463
465
485
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z
O
CH3
OH
N
Br
CH3
N

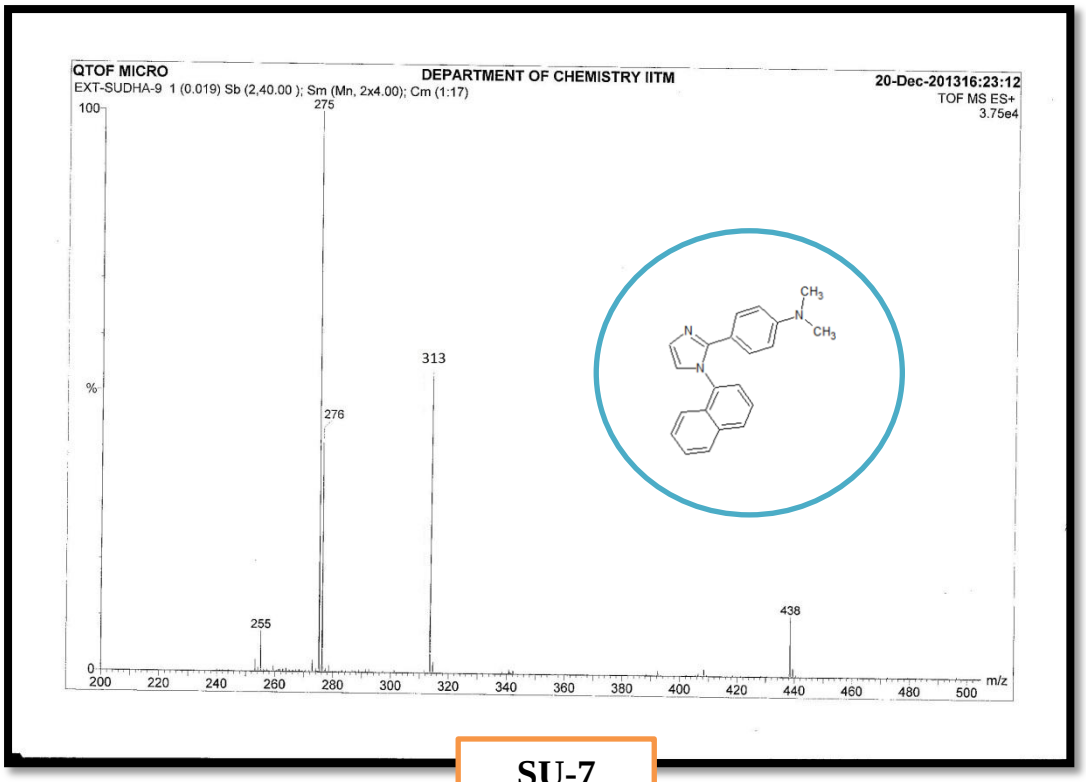
SU-4


QTOF MICRO
EXT-SUDHA-7 85 (1.589) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (40:173)
DEPARTMENT OF CHEMISTRY IITM
20-Dec-201316:16:50
TOF MS ES+
1.24e4
100
%
0
301
297
244
242
241
246
337
203
225
240
238
269
208
215
261
262
248
275
284
293
303
317
319
331
333
302
485
486
487
499
489
502
200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
m/z
N
N
NH
O
N+
O-

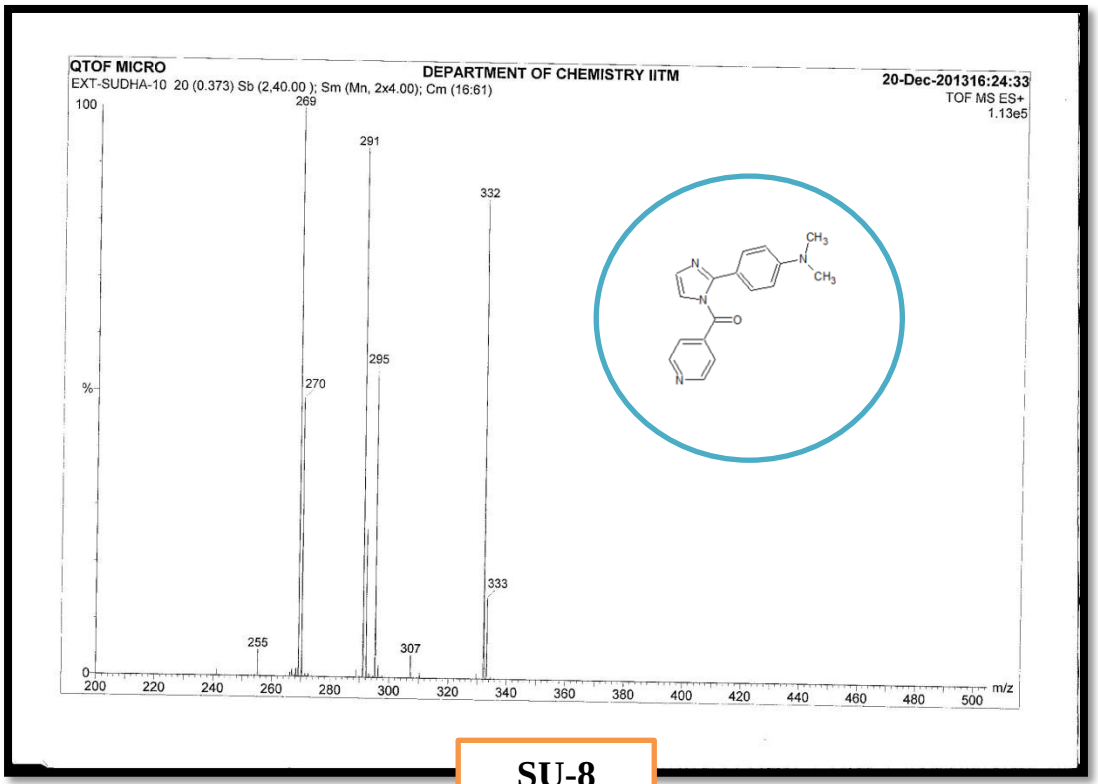
SU-5


QTOF MICRO
EXT-SUDHA-8 4 (0.075) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (4:34)
DEPARTMENT OF CHEMISTRY IITM
20-Dec-201316:21:28
TOF MS ES+
7.35e4
100
%
0
255
254
283
256
253
269
267
295
403
404
416
200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
m/z
CH3
N
CH3
N
N
SH

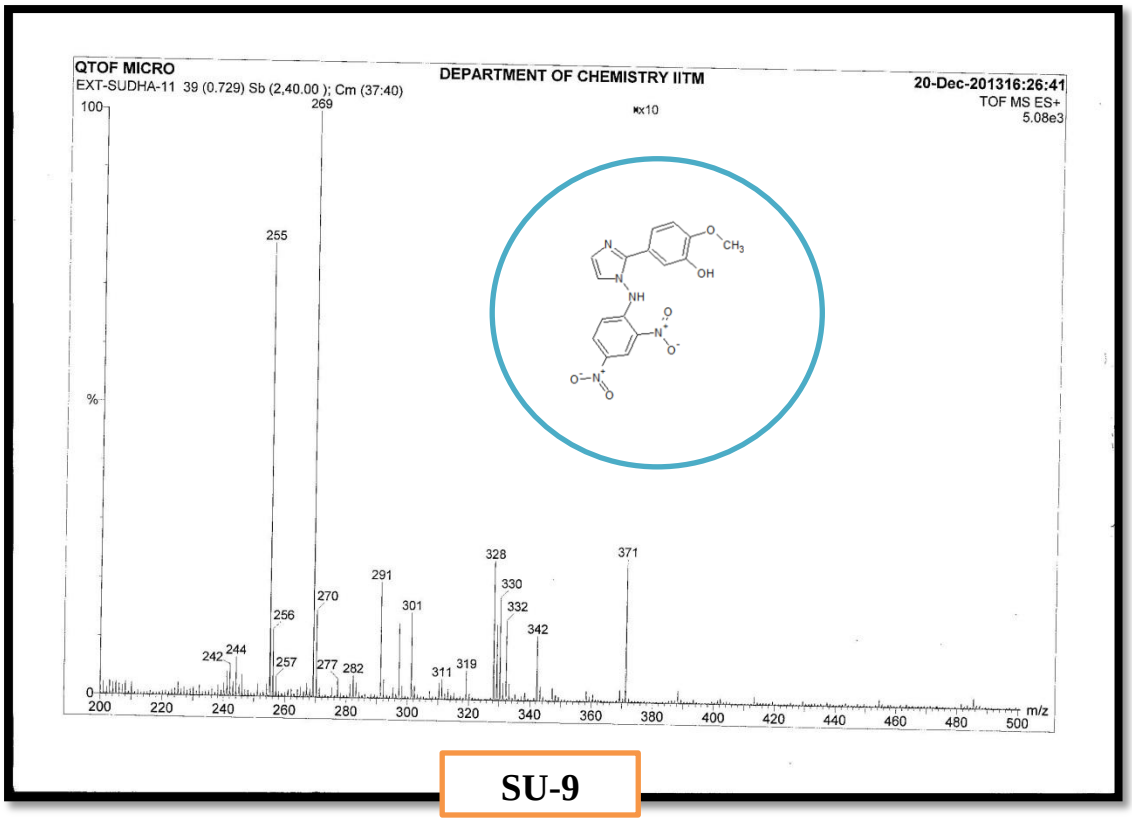
SU-6


QTOF MICRO
DEPARTMENT OF CHEMISTRY IITM
EXT-SUDHA-9 1 (0.019) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (1:17)
20-Dec-201316:23:12
TOF MS ES+
3.75e4
100
%
0
275
313
276
255
438
200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
m/z
CH3
N
CH3
N
N

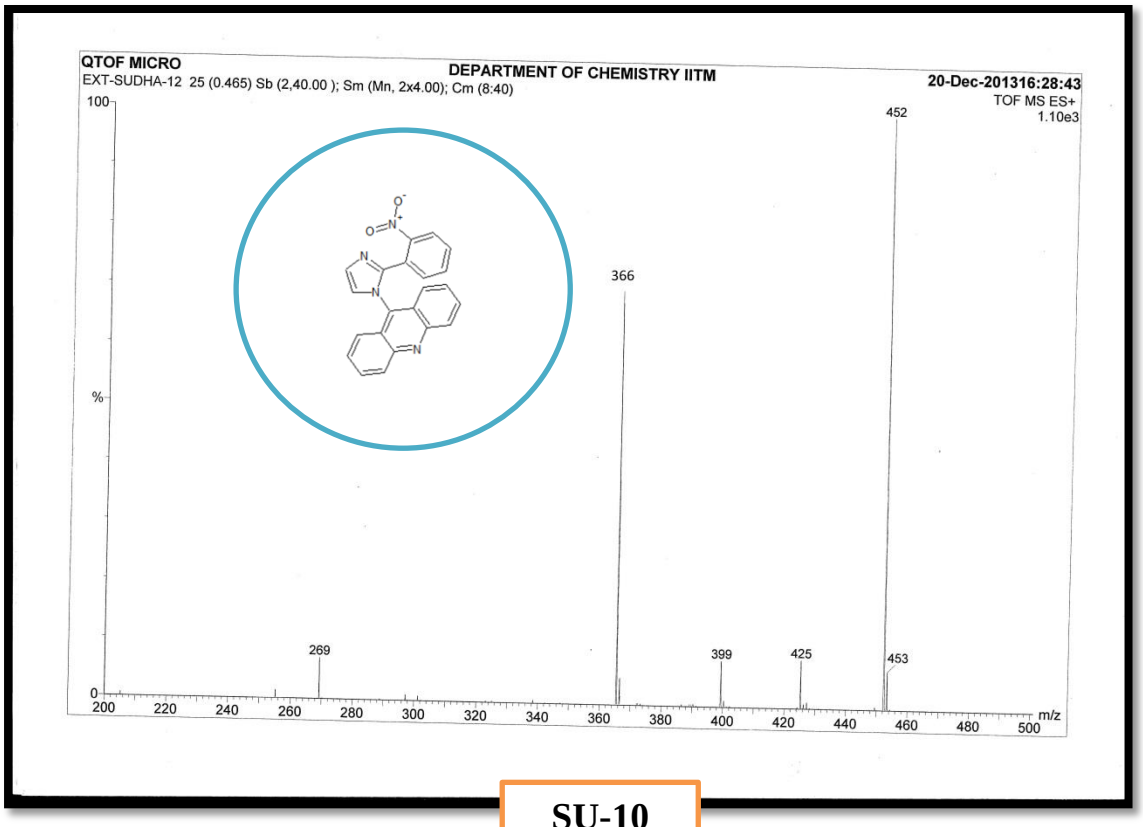
SU-7


QTOF MICRO
DEPARTMENT OF CHEMISTRY IITM
EXT-SUDHA-10 20 (0.373) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (16:61)
20-Dec-201316:24:33
TOF MS ES+
1.13e5
100
%
0
269
291
332
295
270
333
255
307
200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
m/z
CH3
N
CH3
N
N
O
N

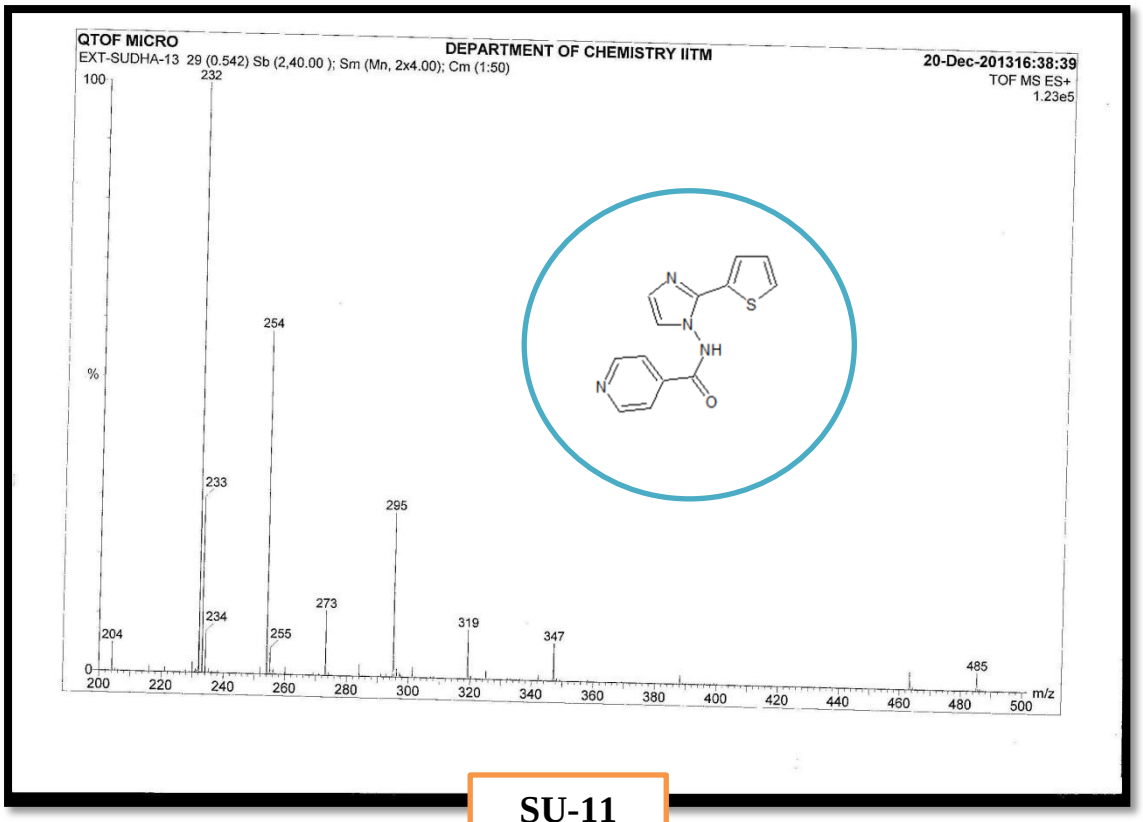
SU-8


QTOF MICRO
EXT-SUDHA-11 39 (0.729) Sb (2,40.00); Cm (37:40)
DEPARTMENT OF CHEMISTRY IITM
20-Dec-201316:26:41
TOF MS ES+
5.08e3
100
%
0
269
255
256
270
291
301
328
330
332
342
371
242
244
257
277
282
311
319
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z

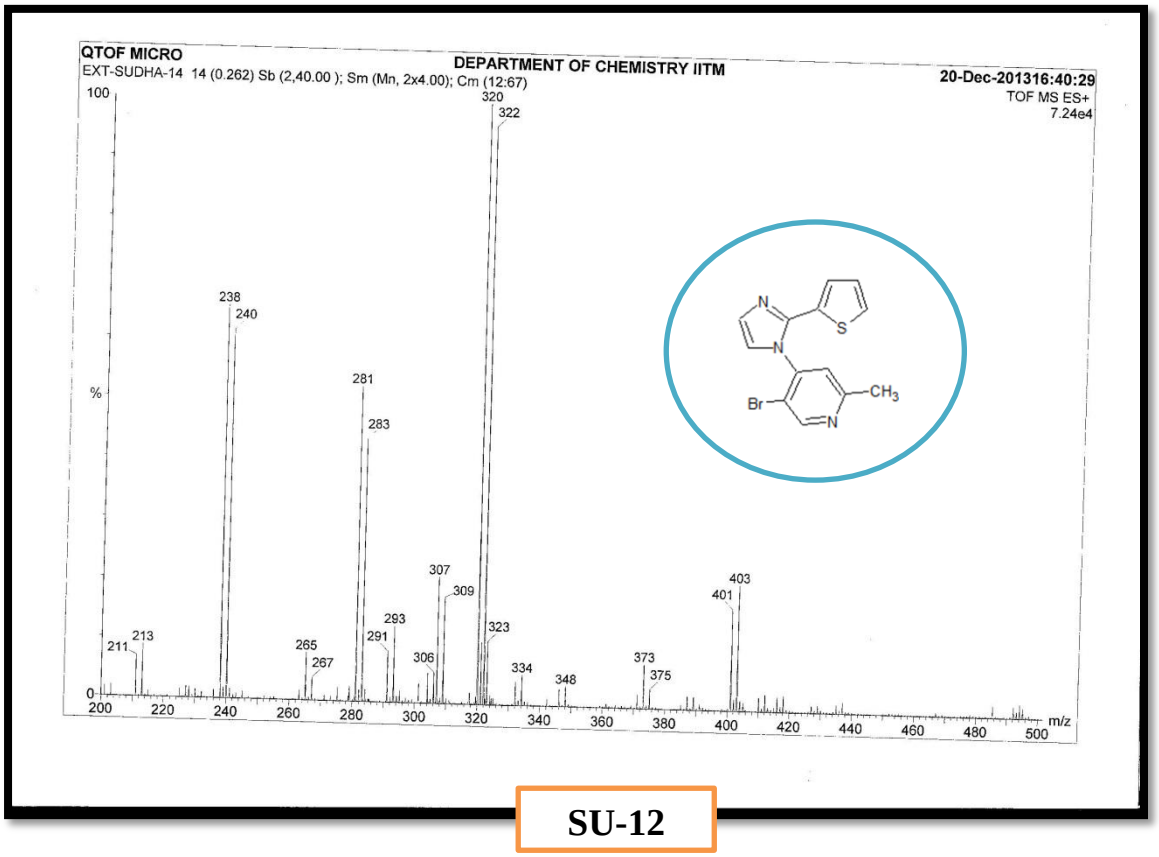
SU-9


QTOF MICRO
EXT-SUDHA-12 25 (0.465) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (8:40)
DEPARTMENT OF CHEMISTRY IITM
20-Dec-201316:28:43
TOF MS ES+
1.10e3
100
%
0
452
366
269
399
425
453
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z

SU-10


QTOF MICRO
EXT-SUDHA-13 29 (0.542) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (1:50)
DEPARTMENT OF CHEMISTRY IITM
20-Dec-201316:38:39
TOF MS ES+
1.23e5
100
%
0
232
254
233
295
273
234
204
255
319
347
485
200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
m/z
N
S
N
NH
N
O

SU-11


QTOF MICRO
EXT-SUDHA-14 14 (0.262) Sb (2,40.00); Sm (Mn, 2x4.00); Cm (12:67)
DEPARTMENT OF CHEMISTRY IITM
20-Dec-201316:40:29
TOF MS ES+
7.24e4
100
%
0
320
322
238
240
281
283
307
309
403
401
293
291
323
211
213
265
267
306
334
348
373
375
200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
m/z
N
S
N
Br
CH3
N

SU-12

IN-VITRO ANTI-TUBERCULAR ACTIVITY

- Compounds were screened for *invitro* anti-tubercular activity (50, 100 µg/ml) by Microplate Alamar Blue Assay (MABA).
- The anti-mycobacterial activities of compounds were assessed against M. tuberculosis using Microplate Alamar Blue Assay (MABA).
- This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.

STANDARD DRUG PHOTOGRAPH (MABA)

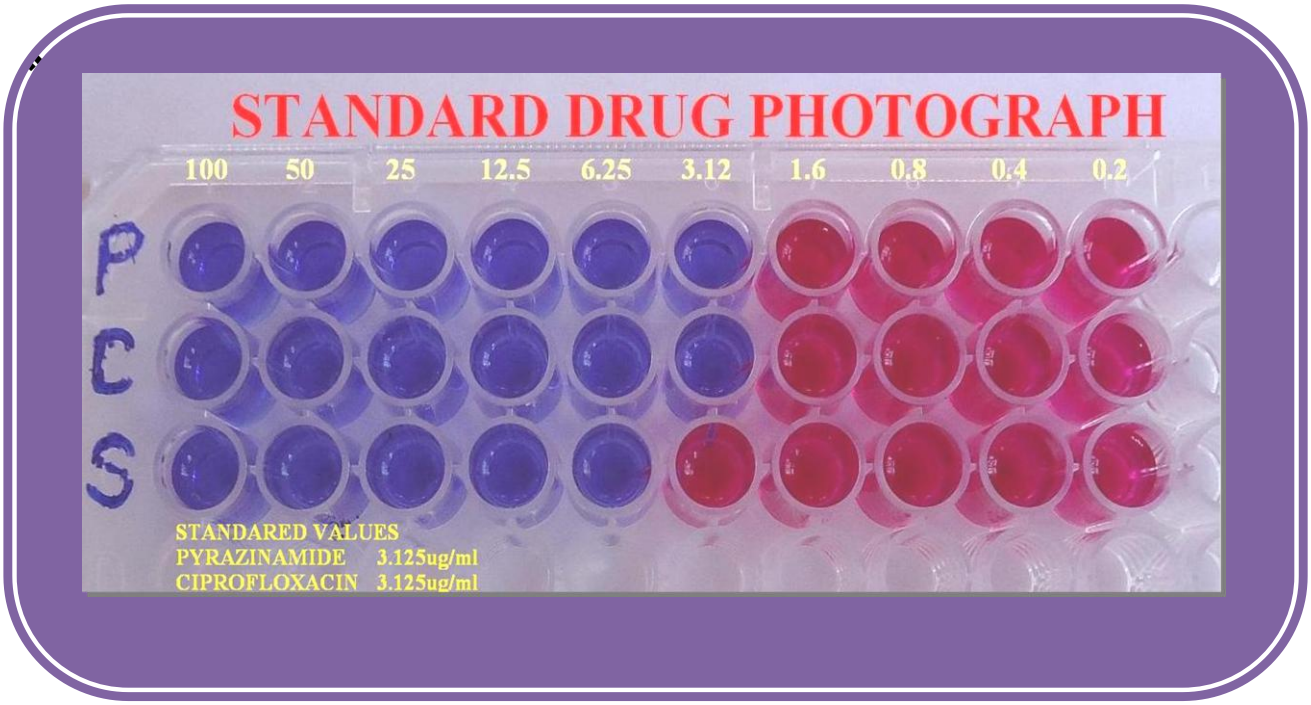


Figure-13

The stranded drug for MABA using pyrazinamide and ciprofloxacin and streptomycin. The minimum inhibitory concentration of pyrazinamide and ciprofloxacin shows 3.12micro gram/ml another stranded drug of streptomycin shows 6.25 micro gram/ml.

IMIDAZOLE NOVEL COMPOUND FROM 1 TO 12(MABA)

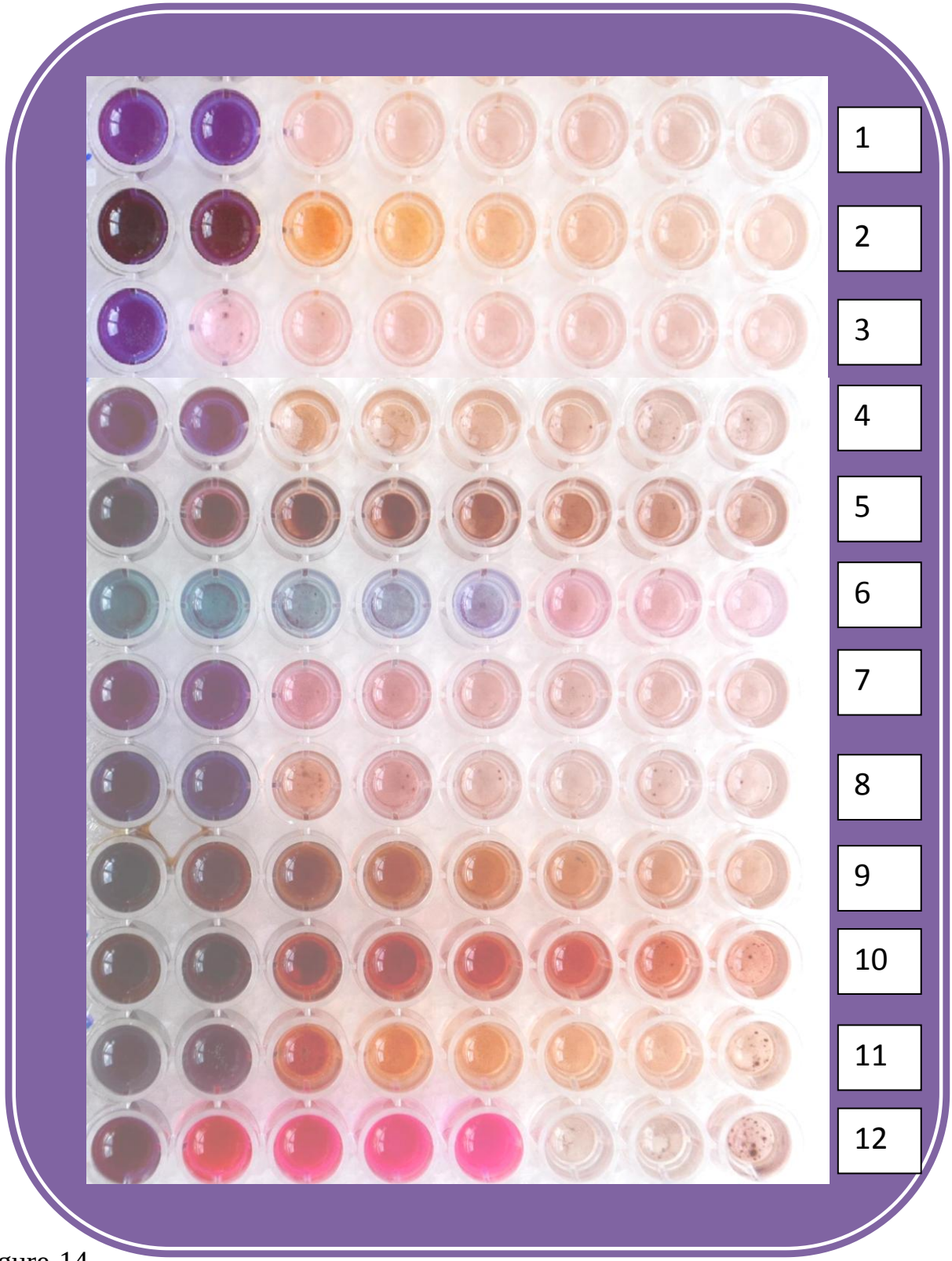


Figure-14

ANTI-TB RESULTS-Table-13

Sl. No.	Sample code	100 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	S	S	R	R	R	R	R	R
2	S	S	R	R	R	R	R	R
3	S	S	R	R	R	R	R	R
4	S	S	R	R	R	R	R	R
5	S	S	R	R	R	R	R	R
6	S	S	R	R	R	R	R	R
7	S	S	R	R	R	R	R	R
8	S	S	R	R	R	R	R	R
9	S	S	R	R	R	R	R	R
10	S	S	R	R	R	R	R	R
11	S	S	R	R	R	R	R	R
12	S	S	R	R	R	R	R	R

S-sensitive

R-resistance

All the 12 compounds exhibited good anti-tubercular activity with range of 50µg/ml. So Compounds SU1-SU12 was found to exhibit moderate anti-tubercular activity.

Summary and conclusion

SUMMARY

- A target critical for the growth of Mycobacterium tuberculosis i.e., 1KPI was chosen.
- A database of 100 molecules with high probability of inhibiting the target 1KPI were chosen by making changes to a known inhibitor scaffold that is aryl substituted Imidazole
- Docking of the 3D structure of these 100 entities against the 3D structure of 1KPI gave an insight about the energetics (molecular docking).
- Of those 100 structures, only 12 structures which showed minimum binding energy were chosen for synthesis.
- The purity of the synthesised compounds were confirmed by TLC and melting point and then characterized by IR, H^1 NMR, C^{13} NMR and Mass spectroscopy.
- The Pure compounds were screened for in-vitro Anti tubercular activity by Microplate Alamar blue Assay method.
- The entire 12 synthesised compound showed sensitivity (Minimum Inhibitory Concentration) 50mcg/ml.
- Toxicity risk assessment prediction was done for all the 12 compounds by Osiris property explorer developed by Acetlion Pharmaceuticals limited available online.

CONCLUSION

All the 12 compounds gave G-score between -6.04to-10.00 kcal/mol. Pyrazinamide gave G-score of -5.6 for KPI, streptomycin gave G-score of -7.4 for KPI and ciprofloxacin gave G-score of -5.9 for KPI .there is correlation between the score and activities of all the 12 compounds which were tested and compared with the standard drugs. This goes to prove that 1KPI is a critical enzyme for anti-mycobacterial activity.so the fine tuning the structures of these 12compoundswill yield molecules with better antimycobacerial activity.

Future scope of study

FUTURE SCOPE OF STUDY

The synthesized compounds should significant anti-tubercular activity in MABA assay method. Hence the anti-tubercular study would deserve for further investigations of in-vivo toxicity and in-vivo anti-tubercular studies.

References

Bibliography

1. Kumar v, abbas ak, fausto n, mitchell rn (2007). Robbins basic pathology (8th ed.). Saunders elsevier. Pp. 516–522. Isbn 978-1-4160-2973-1.

2. Greenblatt cl, editor. Digging for pathogens: ancient emerging diseases: their evolutionary, anthropological and archeological context. Jerusalem: balaban publishers; 1998.

3. Banerjee a, dubnau e, quemard a, balasubramanian v, um ks, wilson t., et al. Inha, a gene coding a target for isoniazid and ethionamide in mycobacterium tuberculosis. Science 1994; 263:227-30.

4. Konstantinos a (2010). Tuberculosis". Australian prescriber **33** (1): 12–18.

5. "tuberculosis". World health organization. 20010

6. Steenken, w., jr., and l. U. Gardner. 1946. History of h37 strain of tubercle Bacillus. Am. Rev. Tuberc. 54:62-66.

7. Thikry, j. P., and a. Rambourg. 1974. Cytochimie des polysaccharides. J. Microsc. 21:225-232.

8. Feizabadi, m. M., i. D. Robertson, d. V. Cousins, and d. J. Hampson. 1996. Genomic analysis of mycobacferium bovis and other members of the mycobacterium tuberculosis complex by isoenzyme analysis and pulsed-field gel electrophoresis. J. Clin. Microbial. 341136-1142.

9. Baffe, m., c. Lacave, m.-a. Laneelle, and g. Laneelle. 1987. Structure of the major triglycosyl phenol-phthiocerol of mycobacteriurn tuberculosis (strain canetti). Eur. J. Biochem. 162155-160.

10. Hermans, p. W. M., d. Van soolingen, e. M. Bik, p. E. W. De haas, j. W.dale, and j. D. A. Van embden. 1991. Insertion element is987 from mycobacterium bovis bcg is located in a hot-

spot integration region for insertion elements in mycobacterium tuberculosis complex strains. Infect. Immun. 5 92695-2705.

11. Wayne, l. G., and g. P. Kubica. 1986. The mycobacteria, p. 1435-1457. In p. H. A. Sneath and j. G. Holt (ed.), bergey's manual of systematic bacteriology, vol. 2. The williams & wilkins co., baltimore, md.

12. Wells, a. Q. 1945. The murine type of tubercle bacillus. Medical research council, sir william dunn school of pathology, university of oxford, oxford,united kingdom. (special report series no. 259.

13. Thoen, c. O., a. G. Karlson, and e. M. Himes. 1984. Disease in domestic andferal animals, p. 1209-1236. In g. 0. Kubica and l. G. Wayne (ed.), the mycobacteria: a sourcebook, part b. Marcel dekker, new york, n.y.

14. Baess, i. 1979. Deoxyribonucleic acid relatedness among species of slowlygrowingn mycobacteria. Acta pathol. Microbiol. Scand. 87221-226.

15. Kolman cj, tuross n ancient dna analysis of human populations. Am j phys anthropol 2000; 111:5-23

16. Rothschild bm, martin ld, lev g et al. (august 2001)."mycobacterium tuberculosis complex dna from an extinct bison dated 17,000 years before the present". Clin. Infect. Dis. 33 (3): 305–11. Doi:10.1086/321886.

17. New delhi: allied chambers india ltd. 1998. P. 352. Isbn 978-81-86062-25-8.

18. Zur pathogenie der impetigines. Auszug aus einer brieflichen mitteilung an den herausgeber. [müller's] archiv für anatomie, physiologie und wissenschaftliche medicin. 1839, page 82.

19. Kapur v, whittam ts, musser jm. Is mycobacteriumtuberculosis 15,000 years old? J infect dis 1994;170:1348–9.

20.crube´zy e´, ludes b, poveda j-d, clayton j, crouau-roy b,montagnon d. Identification of mycobacterium dna in anegyptian pott's disease of 5400 years old. C r acad sci paris (sciences de la vie) 1998;321:941–51

21. Roberts ca, buikstra je. The bioarchaeology of tuberculosis.a global view on a reemerging disease. Gainesville, fl:university of florida press; 2003.

.22. Wilson lg. The historical decline of tuberculosis in europeand america: its causes and significance. J hist med alliedsci 1990; 45:366–96

23. Dubos r, dubos j. Tuberculosis, man, and society. The white plague. Boston, ma: little, brown, and company; 1952.

24. Daniel tm. Rene´ theophile hyacinthe laennec and thefounding of pulmonary medicine.int j tuberc lung dis 2004; 8:517–8.

25. Davies tw, davies m. Mass radiography in wales. Experience with a mobile unit. Br j tuberc dis chest 1945; 391:2–37.

26. Hinshaw hv, feldman wh. Streptomycin in the treatment ofclinical tuberculosis: a preliminary report. Proc staff meetingsmayo clinic 1945; 20:313–8.

27. Streptomycin treatment of pulmonary tuberculosis. Amedical research council investigation. Brit med j 1948; 2:769–82.

28. Weitzman d, de wend cayley fe, wingfield al. Streptomycinin the treatment ofpulmonary tuberculosis. Brit j tuberc dischest 1950; 44:98–104.

29. Menzies d, pai m, and comstock g. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann intern med. 2007; 146:340–354

.

30. Kaplan je, benson c, holmes kh, brooks jt, pau a, et al. Guidelines for prevention and treatment of opportunistic infections in hiv-infected adults and adolescents: recommendations from cdc, the national institutes of health, and the hiv medicine association of the infectious diseases society of america. Mmwr recomm rep. 2009; 58:1–207; quiz ce201–204.

31. Pozniak al, coyne km, miller rf, lipman mci, freedman ar, et al. British hiv association guidelines for the treatment of tb/hiv coinfection 2010. 2010. Available:http://www.bhiva.org/documents/guidelines/tb/110204cons-tb_coinfection_guideline.pdf. Accessed 15 july 2011.

32. Aichelburg mc, rieger a, breitenecker f, pfistershammer k, tittes j, et al. Detection and prediction of active tuberculosis disease by a whole-blood interferon-gamma release assay in hiv-1-infected individuals. Clin infect dis. 2009; 48:954–962.

33. Tan ck, hung cc, lai cc, liao ch, chou ch, et al. Diagnosis of active tuberculosis by enzyme-linked immunospot assay for interferon-gamma in hiv-infected patients. J acquir immune defic syndr. 2010; 53:546–547

34. Sester m, sotgiu g, lange c, giehl c, girardi e, et al. Interferon-gamma release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. Eur respir j. 2011; 37:100–111.

35. Moher d, liberati a, tetzlaff j, altman dg. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. Ann intern med. 2009;151:w264.

36. Whiting p, rutjes aw, reitsma jb, bossuyt pm, kleijnen j. The development of quadas: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. Bmc med res methodol. 2003;3:25.

37. Whiting pf, weswood me, rutjes aw, reitsma jb, bossuyt pn, et al. Evaluation of quadas, a tool for the quality assessment of diagnostic accuracy studies. Bmc med res methodol. 2006;6:9.

38. Zamora j, abraira v, muriel a, khan k, coomarasamy a. Meta-disc: a software for meta-analysis of test accuracy data. Bmc med res methodol. 2006;6:31.

39. Aabye mg, ravn p, praygod g, jeremiah k, mugomela a, et al. The impact of hiv infection and cd4 cell count on the performance of an interferon gamma release assay in patients with pulmonary tuberculosis. Plos one. 2009;4:e4220.

40. Aabye mg, ruhwald m, praygod g, jeremiah k, faurholt-jepsen m, et al. Potential of interferon-gamma-inducible protein 10 in improving tuberculosis diagnosis in hiv-infected patients. Eur respir j.2010;36:1488–1490.

41. Garcia-gasalla m, fernandez-baca v, mir-viladrich i, cifuentes-luna c, campins-rosello a, et al. [quantiferon-tb gold in-tube test in the diagnosis of pulmonary and extra-pulmonary tuberculosis].enferm infecc microbiol clin. 2010;28:685–689.

42. Syed ahamed kabeer b, sikhamani r, swaminathan s, perumal v, paramasivam p, et al. Role of interferon gamma release assay in active tb diagnosis among hiv infected individuals. Plos one.2009;4:e5718.

43. Kabeer bs, sikhamani r, raja a. Comparison of interferon gamma and interferon gamma-inducible protein-10 secretion in hiv-tuberculosis patients. Aids. 2010;24:323–325.

44. Legesse m, ameni g, mamo g, medhin g, bjune g, et al. Performance of quantiferon-tb gold in-tube (qftgit) for the diagnosis of mycobacterium tuberculosis (mtb) infection in afar pastoralists, ethiopia. Bmc infect dis. 2010;10:354.

45. Ling di, pai m, davids v, brunet l, lenders l, et al. Are interferon-{gamma} release assays useful for active tuberculosis in a high-burden setting? Eur respir j 2011

46. Rangaka mx, gideon hp, wilkinson ka, pai m, mwansa-kambafilwe j, et al. No discriminatory value of interferon release added to smear negative hiv-tuberculosis algorithms. Eur respir j. 2011.

47. Barry ce 3rd, et al. Mycolic acids: structure, biosynthesis and physiological functions. Prog lipid
Res 1998;37:143–179.

48. Hong x, hopfinger aj. Construction, molecular modeling, and simulation of mycobacterium Tuberculosis cell walls. Biomacromolecules 2004;5:1052–1065.

49. Villeneuve m, et al. Temperature dependence of the langmuir monolayer packing of mycolic acids

From mycobacterium tuberculosis. Biochim biophys acta 2005; 1715:71–80.

50. Villeneuve m, et al. Conformational behavior of oxygenated mycobacterial mycolic acids frommycobacterium bovis bcg. Biochim biophys acta 2007;1768:1717–1726.

51. Glickman ms, et al. A novel mycolic acid cyclopropane synthetase is required for cording, Persistence, and virulence of mycobacterium tuberculosis. Mol cell 2000;5:717–727.

52. Rao v, et al. Trans-cyclopropanation of mycolic acids on trehalose dimycolate suppresses Mycobacterium tuberculosis -induced inflammation and virulence. J clin invest 2006;116:1660–1667.

53. Bhatt a, et al. Loss of a mycobacterial gene encoding a reductase leads to an altered cell wall Containing beta-oxo-mycolic acid analogs and accumulation of ketones. Chem biol2008;15:930–939.

54. Liu j, nikaido h. A mutant of mycobacterium smegmatis defective in the biosynthesis of mycolic acids accumulates meromycolates. Proc natl acad sci usa 1999;96:4011–4016.

55. Wang l, et al. Cell wall structure of a mutant of mycobacterium smegmatis defective in the Biosynthesis of mycolic acids. J biol chem 2000;275:7224–7229.

56. Portevin d, et al. A polyketide synthase catalyzes the last condensation step of mycolic acid Biosynthesis in mycobacteria and related organisms. Proc natl acad sci usa 2004;101:314–319

57. Brennan pj, nikaido h. The envelope of mycobacteria. Annu rev biochem 1995;64:29–63.

58. Minnikin, de. Lipids: complex lipids, their chemistry, biosynthesis and roles. In: ratledge, c.;stanford, j., editors. The biology of the mycobacteria: physiology, identification and classification.academic press; 1982. P. 95-184.

59. Rastogi n, et al. The mycobacteria: an introduction to nomenclature and pathogenesis. Rev sci tech
2001;20:21–54

60. Barry ce. Interpreting cell wall 'virulence factors' of mycobacterium tuberculosis. Trends microbiol
2001;9:237–241.

61. Nikaido h, et al. Physical organization of lipids in the cell wall of mycobacterium chelonae. Molmicrobiol 1993;8:1025–1030.

62. Minnikin de, et al. The methyl-branched fortifications of mycobacterium tuberculosis. Chem biol
2002;9:545–553.

63. Bhamidi s, et al. The identification and location of succinyl residues and the characterization of theinterior arabinan region allows for a model of the complete primary structure of mycobacteriumtuberculosis mycolyl arabinogalactan. J biol chem 2008;283:12992–13000.

64. Trias j, et al. Porins in the cell wall of mycobacteria. Science 1992;258:1479–1481.

65. Niederweis m, et al. Cloning of the mspa gene encoding a porin from mycobacterium smegmatis. Molmicrobiol 1999;33:933–945

66. Faller m, et al. The structure of a mycobacterial outer-membrane channel. Science 2004;303:1189–1192.

67. Recht j, kolter r. Glycopeptidolipid acetylation affects sliding motility and biofilm formation inmycobacterium smegmatis. J bacteriol 2001;183:5718–572468. Schulze-röbbecker, fischeder r. Mycobacteria in biofilms. Zentralbl hyg umweltmed
1989;188:385–390.

69. Ojha a, et al. Groel1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilmformation in mycobacteria. Cell 2005;123:861–873.

70. Collins rf, derrick jp. Wza: a new structural paradigm for outer membrane secretory proteins?trends microbiol 2007;15:96–100.

71. Dong c, et al. Wza, the translocon for e. Coli capsular polysaccharides defines a new class of Membrane protein. Nature 2006;444:226–229.

72. Digiuseppe champion pa, cox js. Protein secretion systems in mycobacteria. Cell microbiol2007;9:1376–1384.

73. Simeone r, et al. Esx/type vii secretion systems and their role in host-pathogen interaction. Curropin microbiol 2009;12:4–10.

74. Raynaud c, et al. Phospholipases c are involved in the virulence of mycobacterium tuberculosis.mol microbiol 2002;45:203–217.

75. Lun s, bishai wr. Characterization of a novel cell wall-anchored protein with carboxylesteraseactivity required for virulence in mycobacterium tuberculosis. J biol chem 2007;282:18348–18356.

76. Cascioferro a, et al. Pe is a functional domain responsible for protein translocation and localization
On mycobacterial cell wall. Mol microbiol 2007;66:1536–1547.

77. Niederweis m. Nutrient acquisition by mycobacteria. Microbiology 2008;154:679–692.

78. Schnappinger d, et al. Transcriptional adaptation of mycobacterium tuberculosis within Macrophages: insights into the phagosomal environment. J exp med 2003;198:693–704.

79. Munoz-elias ej, mckinney jd. Mycobacterium tuberculosis isocitrate lyases 1 and 2 are jointlyrequired for in vivo growth and virulence. Nat med 2005;11:638–644.

80. Neyrolles o, et al. Is adipose tissue a place for mycobacterium tuberculosis persistence? Plos one
2006;1:e43.

81. Joshi sm, et al. Characterization of mycobacterial virulence genes through genetic interactionmapping. Proc natl acad sci usa 2006;103:11760–11765.

82. Van der geize r, et al. A gene cluster encoding cholesterol catabolism in a soil actinomycete providesinsight into mycobacterium tuberculosis survival in macrophages. Proc natl acad sci usa2007;104:1947–1952.

83. Pandey ak, sassetti cm. Mycobacterial persistence requires the utilization of host cholesterol. Pronatl acad sci usa 2008;105:4376–4380.

84. Nikaido h. Preventing drug access to targets: cell surface permeability barriers and active efflux inbacteria. Semin cell dev biol 2001;12:215–223.

85. De rossi e, et al. Role of mycobacterial efflux transporters in drug resistance: an unresolved question.fems microbiol rev 2006;30:36–52.

86. Li xz, nikaido h. Efflux-mediated drug resistance in bacteria. Drugs 2004;64:159–204.

87. Sulavik mc, et al. Antibiotic susceptibility profiles of escherichia coli strains lacking multidrugefflux pump genes. Antimicrob agents chemother 2001;45:1126–1136.

88. Danilchanka o, et al. Identification of a novel multidrug efflux pump of mycobacterium Tuberculosis. Antimicrob agents chemother 2008;52:2503–2511.

89. Etienne g, et al. The cell envelope structure and properties of mycobacterium smegmatis mc(2)155:is there a clue for the unique transformability of the strain? Microbiology 2005;151:2075–2086. [pubmed: 15942014]

90. Paul tr, beveridge tj. Reevaluation of 11. Niederweis m. Mycobacterial porins - new channel proteins in unique outer membranes. Mol
Microbiol 2003;49:1167–1177.

91. Mahfoud m, et al. Topology of the porin mspa in the outer membrane of mycobacterium Smegmatis. J biol chem 2006;281:5908–5915.

92. Chatterjee d. The mycobacterial cell wall: structure, biosynthesis and sites of drug action. Curr opin chem biol 1997;1:579–588.

93. Dover lg, et al. Comparative cell wall core biosynthesis in the mycolated pathogens, mycobacterium tuberculosis and corynebacterium diphtheriae. Fems microbiol rev 2004;28:225–250.

94. Puech v, et al. Structure of the cell envelope of corynebacteria: importance of the non-covalentlybound lipids in the formation of the cell wall permeability barrier and fracture plane. Microbiology2001;147:1365–1382

95. Etienne g, et al. The cell envelope structure and properties of mycobacterium smegmatis mc(2)155:is there a clue for the unique transformability of the strain? Microbiology 2005;151:2075–2086.

96. Paul tr, beveridge tj. Reevaluation of envelope profiles and cytoplasmic ultrastructure of Mycobacteria processed by conventional embedding and freeze-substitution protocols. J bacteriol1992;174:6508–6517.

97. Hoffmann c, et al. Disclosure of the mycobacterial outer membrane: cryo-electron tomography and vitreous sections reveal the lipid bilayer structure. Proc natl acad sci u s a 2008;105:3963–3967.

98. Zuber b, et al. Direct visualization of the outer membrane of native mycobacteria and corynebacteria.j bacteriol 2008;190:5672–5680. [pubmed: 18567661]

100. Matias vr, et al. Cryo-transmission electron microscopy of frozen-hydrated sections of escherichiacoli and pseudomonas aeruginosa. J bacteriol 2003;185:6112–6118.

101. Nikaido h. Molecular basis of bacterial outer membrane permeability revisited. Microbiol mol biolrev 2003;67:593–656.

102.butler tz, et al. Single-molecule dna detection with an engineered mspa protein nanopore. Procnatl acad sci u s a 2008;105:20647–20652.

103. Basel mt, et al. Direct observation of gold nanoparticle assemblies with the porin mspa on mica.acs nano 2009;3:462–466.

104. Wörner m, et al. Characterization of nanostructured surfaces generated by reconstitution of the porinmspa from mycobacterium smegmatis. Small 2007;3:1084–1097

105. Schulz ge. Transmembrane beta-barrel proteins. Adv protein chem 2003;63:47–70.

106. Niederweis, m. Mycobacterial porins. In: daffe, m.; reyrat, jm., editors. The mycobacterial cellenvelope. Asm press; 2008. P. 153-165.

107. Postle k, larsen ra. Tonb-dependent energy transduction between outer and cytoplasmic Membranes. Biometals 2007;20:453–465.

108. Miethke m, marahiel ma. Siderophore-based iron acquisition and pathogen control. Microbiol molbiol rev 2007;71:413–451.

109. Braun v. Iron uptake by escherichia coli. Front biosci 2003;8:s1409–1421.

110. Ratledge c, dover lg. Iron metabolism in pathogenic bacteria. Annu rev microbiol 2000;54:881–941

111. Rodriguez gm, smith i. Identification of an abc transporter required for iron acquisition andvirulence in mycobacterium tuberculosis. J bacteriol 2006;188:424–430.

112. Danilchanka o, et al. Identification of a novel multidrug efflux pump of mycobacterium Tuberculosis. Antimicrob agents chemother 2008;52:2503–2511.

113. Doerrler wt, raetz cr. Loss of outer membrane proteins without inhibition of lipid export in anescherichia coli yaet mutant. J biol chem 2005;280:27679–27687.

114. Werner j, misra r. Yaet (omp85) affects the assembly of lipid-dependent and lipid-independentouter membrane proteins of escherichia coli. Mol microbiol 2005;57:1450–1459.

115. Voulhoux r, et al. Role of a highly conserved bacterial protein in outer membrane protein assembly.science 2003;299:262–265.

116. Wu t, et al. Identification of a protein complex that assembles lipopolysaccharide in the outermembrane of escherichia coli. Proc natl acad sci usa 2006;103:11754–11759.

117. Camacho lr, et al. Identification of a virulence gene cluster of mycobacterium tuberculosis bysignature-tagged transposon mutagenesis. Mol microbiol 1999;34:257–267.

118. Cox js, et al. Complex lipid determines tissue-specific replication of mycobacterium tuberculosis inmice. Nature 1999; 402:79–83.

119.cooper, a. Cell mediated immune responses in tuberculosis. Ann rev immunol (2009). , 27, 393-422.

120.wolf, a, desvignes, l, linas, b, banaiee, n, tamura, t, takatsu, k, & ernst, j. Initiation Of the adaptive immune response to mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs. J exp med (2008). , 205, 105-115.

121.reiley, w, calayag, m, wittmer, s, huntington, j, pearl, j, fountain, j, martino, c, Roberts, a, cooper, a, winslow, g, et al. Esat-6-specific cd4 t cell responses to Aerosol mycobacterium tuberculosis infection are initiated in mediastinal lymph nodes. Proc natl acad sci usa (2008). , 105, 10961-10966.

122.ngai, p, mccormick, s, small, c, zhang, x, zganiacz, a, aoki, n, & xing, z. Gamma Interferon responses of cd4 and cd8 t-cell subsets are quantitatively different and Independent of each other during pulmonary mycobacterium bovis bcg infection. Infect Immun (2007). , 75, 2244-2252.

123.herrera, m. T, torres, m, nevels, d, et al. Compartmentalized bronchoalveolar ifngamma And il-12 response in human pulmonary tuberculosis. Tuberculosis (edinb) (2009). , 89, 38-47.

124. Ordway, d. J, & orme, i. M. Animal models of mycobacteria infection. Current protocolsin immunology (2011). Supple , 94, 1-50.

125. Mcmurray, d. N. Disease model: pulmonary tuberculosis. Trends mol med (2001). , 7, 135-137.

126. Mitchison, d. A. The diagnodis and therapy of tuberculosis during the past 100 Years. Am j respir crit care med (2005). , 171, 699-706.

127. Sugawara, i, yamada, h, & mizuno, s. Pathological and immunological profiles of Rat tuberculosis. Int j exp pathol (2004). , 85, 125-134.

128. Mcmurray, d. N. Guinea pig model of tuberculosis. Washington dc: american society For microbiology, (1994).

129. Ordway, d, palanisamy, g, henao-tamayo, m, et al. The cellular immune response to Mycobacterium tuberculosis infection in the guinea pig. J immunol (2007). , 179, 2532-2541.

130.woolard, m. D, hudig, d, tabor, l, ivey, j. A, & simecka, j. W. Nk cells in gammainterferon-deficient mice suppress lung innate immunity against mycoplasma spp. Infect immun (2005). , 73, 6742-6751.

131. Goren mb (1977) phagocyte lysosomes: interactionswith infectious agents, phagosomes, and experimentalperturbations in function. Annu rev microbiol 31, 507–533.

132 gorden ah, hart pa & young mr (1980) ammoniainhibits phagosomes-lysosome fusion in macrophages.nature 286, 79–81.3

.133. Fortune, s. M., a. Jaeger, d. A. Sarracino, m. R. Chase, c. M. Sassetti, d. R.sherman, b. R. Bloom, and e. J. Rubin. 2005. Mutually dependent secretionof proteins required for mycobacterial virulence. Proc. Natl. Acad. Sci.u. S. A. 102:10676–10681

134.anon. 1976. Annual report of the ministry ofagriculture and forestry resources., northern nigeria (1976).

135. Who 2012. Zoonotic tuberculosis mycobacteriumbovis. Memorandum from a who meeting with the participation of fao. Bull. Who. 72: 851-857

136. Boulle a, van cutsem g, cohen k, et al. Outcomes of nevirapine- and efavirenz-based antiretroviral therapy when coadministered with rifampicin-based antitubercular therapy. Jama 2013; 300: 530–39

137. Duan y, reddy bv, kaznessis yn. Physicochemical and residue conservation calculations to improve the ranking of protein-protein docking solutions. Protein sci.2005;14:316–328

138. Heusear p, bau d, benkert p, schomburg d. Refinement of unbound protein docking studies using biological knowledge. Proteins. 2005;61:1059–1067.

139. Tress m, dejuan d, grana o, gomez mj, gomez-puertas p, gonzalez jm, lopez g, valencia a. Scoring docking models with evolutionary information. Proteins.2005;60:275–280.

140. Chelliah v, blundell tl, fernandez-recio j. Efficient restraints for protein-protein docking by comparison of observed amino acid substitution patterns with those predicted from local environment. J mol biol. 2006;357:1669–1682

141. Comeau sr, gatchell dw, vajda s, camacho cj. Cluspro: an automated docking and discrimination method for the prediction of protein complexes. Bioinformatics.2004;20:45–50.

142. Mendez r, laplae r, lensink mf, wodak sj. Assessment of capri predictions in rounds 3–5 shows progress in docking procedures. Proteins. 2005;60:150–169.

143. Gray jj. High-resolution protein-protein docking. Curr opin struct biol. 2006;16:183–193.

144. Lensink mf, mendez r, wodak sj. Docking and scoring protein complexes: capri 3rd edition. Proteins. 2007;69:704–718

145. Aloy p, querol e, aviles fx, sternberg mj. Automated structure-based prediction of functional sites in proteins: applications to assessing the validity of inheriting protein function from homology in genome annotation and to protein docking. J mol biol.2001;311:395–408.

146. Zhang c, liu s, zhou y. Docking prediction using biological information, zdock sampling technique and clusterization guided by the dfire statistical energy function.proteins. 2005;60:314–318

147. Amari s, aizawa m, zhang j, fukuzawa k, mochizuki y, iwasawa y, nakata k, chuman h, nakano t (2006). "viscana: visualized cluster analysis of protein-ligand interaction based on the ab initio fragment molecular orbital method for virtual ligand screening". J chem inf model**46** (1): 221–30. Doi:10.1021/ci050262q

148.j.k. Belanoff, b.h. Flores, m. Kalezhan, b. Sund, and a.f. Schatzberg, “rapid reversal
Of psychotic depression using mifepristone,” journal of clinical psychopharmacology,
Vol. 21, oct. 2001, pp. 516-521

.

149. P. Shannon, a. Markiel, o. Ozier, n.s. Baliga, j.t. Wang, d. Ramage, n. Amin, b. Schwikowski, and t. Ideker, “cytoscape: a software environment for integrated models of biomolecular interaction networks,” genome research, vol. 13, nov. 2003,pp. 2498-2504.

150. S.d. Hooper and p. Bork, “medusa: a simple tool for interaction graph analysis,” bioinformatics (oxford, england), vol. 21, dec. 2005, pp. 4432-4433.

151.t.c. Freeman, l. Goldovsky, m. Brosch, s. Van dongen, p. Mazière, r.j. Grocock, s. Freilich, j. Thornton, and a.j. Enright, “construction, visualisation, and clustering of transcription networks from microarray expression data,” plos computational biology, vol. 3, oct. 2007, pp. 2032-2042.

152. J.a. Dimasi, r.w. Hansen, and h.g. Grabowski, “the price of innovation: new estimates of drug development costs,” journal of health economics, vol. 22, mar.
2003, pp. 151-185.

153. C. Adams and v. Brantner, “estimating the cost of new drug development: is it really 802 million dollars?,” health affairs (project hope), vol. 25, r. 2006, pp. 428, 420.

154.t. Ashburn and k. Thor, “drug repositioning: identifying and developing new uses for existing drugs,” nature reviews drug discovery, vol. 3, 2004, pp. 683, 673.

155.i. Goldstein, t.f. Lue, h. Padma-nathan, r.c. Rosen, w.d. Steers, and p.a. Wicker, “oral sildenafil in the treatment of erectile dysfunction. Sildenafil study group,” the new england journal of medicine, vol. 338, may. 1998, pp. 1397-1404.

156.k.b. Thor and m.a. Katofiasc, “effects of duloxetine, a combined serotonin and norepinephrine reuptake inhibitor, on central neural control of lower urinary tract function in the chloralose-anesthetized female cat,” j pharmacol exp ther.

157. S. Haider, b. Ballester, d. Smedley, j. Zhang, p. Rice, and a. Kasprzyk, “biomart
Central portal--unified access to biological data.,” nucleic acids research, vol. 37, jul.
2009, pp. 27, w23.

158. A. Prlić, t.a. Down, e. Kulesha, r.d. Finn, a. Kähäri, and t.j.p. Hubbard, "integrating sequence and structural biology with das," bmc bioinformatics, vol. 8, 2007, p. 333.

159. J. Kohler, j. Baumbach, j. Taubert, m. Specht, a. Skusa, a. Ruegg, c. Rawlings, p. Verrier, and s. Philippi, "graph-based analysis and visualization of experimental results With ondex," bioinformatics, vol. 22, jun. 2006, pp. 1390, 1383
.

160. J. Taubert, k.p. Sieren, m. Hindle, b. Hoekman, r. Winnenburg, s. Philippi, c. Rawlings, and j. Kohler, "the oxl format for the exchange of integrated datasets," Journal of integrative bioinformatics, vol. 4, 2007.

161.d. Wishart, c. Knox, a.c. Guo, d. Cheng, s. Shrivastava, d. Tzur, b. Gautam, and m. Hassanali, "drugbank: a knowledgebase for drugs, drug actions and drug targets.," Nucleic acids research, vol. 36, jan. 2008, p. Gkm958.

162. "the universal protein resource (uniprot) 2009.," nucleic acids research, vol. 37,n Jan. 2009, pp. 174, d169.

163. paul, n. Et al. (2004) recovering the true targets of specific ligands by virtual screening of the protein data bank. Proteins 54, 671–680

164. Hofmann, k., hsiao, c.-y. Y., henis, d. B., and pa~os, c., j. Biol. Chem., 217, 49 (1955).

165. Hofmann, k., henis, d. B., and panos, c., j. Biol. Chem., 228, 349 (1957).

166. Liu, t. Y., and hofmann, k., biochemistry, 1, 189 (1962).

167. Hofmann, k., and liu, t. Y., biochim. Et biophys. Acta, 37, 364 (1960).

168. Dauchy, s., and asselineau, j., compt. Rend., 250, 2635(1960).

170. Kaneshiro, t., and marr, a. G., j. Biol. Chem., 236, 2615

171. Dubnau, e., marrakchi, h., smith, i., daffe, m., and quemard, a. (1998) mol. Microbiol. **29,** 1526–1528

172. Yuan, y., zhu, y., crane, d. D., and barry, c. E., iii (1998) mol. Microbiol.

1449–1458cmaa2 encodes a trans-cyclopropane synthetase 22

173.zhao, y.h., le, j., abraham, m.h., hersey, a., eddershaw, p.j., lus- suzuki, a., higuchi, w.i., ho, n.f., 1970a.

174. Poulin, p., theil, f.p., 2000. A priori prediction of tissue:plasma partition de waterbeemd, h., folkers, g., guy, r. (eds.), pharmacokineticof drugs to facilitate the use of physiologically-based optimization in drug research—biological, physicochemical and pharmacokinetic models in drug discovery. J. Pharm. Sci. 89, 16–35

175. Accelrys. Adme-tox component collection. Http://accelrys.com/products/pipeline-pilot/ Component-collections/adme-tox.html

176.g. Dent, j.m. Chalmers, industrial analysis with vibrationalspectroscopy, royal society of chemistry, cambridge,1997.

177.james, t. L. 1975. Nuclear magnetic resonance in biochemistry. Academic press, new york.

178. Barber, m.; bordoli, r.s.; elliott, g.j.; sedgwick, r.d.; tyler, a.n. Anal. Chem. 1982, 54, 645a-657a.

179. Tiballi, r. N., x. He, l. T. Zarins, s. G. Revankar, and c. A. Kauffman. 1995.use of a colorimetric system for yeast susceptibility testing. J. Clin. Microbiol.33:915–917.

180. Wright, e. L., d. C. Quenelle, w. J. Suling, and w. W. Barrow. 1996. Use ofmono mac 6 human monocytic cell line and j774 murine macrophage cellline in parallel antimycobacterial drug studies. Antimicrob. Agents chemother.40:2206–2208.

181. Yajko, d. M., j. J. Madej, m. V. Lancaster, c. A. Sanders, v. L. Cawthon, b.gee, a. Babst, and w. K. Hadley. 1995. Colorimetric method for determiningmics of antimicrobial agents for mycobacterium tuberculosis. J. Clin. Microbiol.33:2324–2327.

182. Zabransky, r. J., a. R. Dinuzzo, and g. L. Woods. 1995. Detection ofvancomycin resistance in enterococci by the alamar mic system. J. Clin.microbiol. 33:791–793.

183. Sakai r, higa t, jefford cw, bernardinelli g (1986) j am chem soc 108:6404–6405

184. . I. Sakiyan, n. Gunduz, and t. Gunduz, react. Inorg., met. Org. Chem., 2001, 31, 1175.

185. D. Zurita, s. Menage, j. L. Pierre, and e. S. Aman, j. Biol. Inorg. Chem., 1997, 2, 46.

186. E. Tas, m. Aslanoglu, m. Guler, and m. Ulusoy, j. Coord. Chem., 2004, 57, 583.

187. Y. Özcan, s. Ide, i. Sakıyan, and e. Logoglu, j. Mol. Struc., 2003, 658, 207.

188.hadizadeh f and ghodsi r. Synthesis of novel n-substituted imidazolecarboxylic acid hydrazides as monoamine oxidase inhibitors. Farmaco (2005) 60: 237-240

189.porsolt rd, bertin a and jalfre m. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. (1977) 229: 327-336

190.cryan jf, markou a and lucki i. Assessing antidepressant activity in rodents: recent developments and future needs. Trends pharmacol. Sci. (2002) 23: 238-245

191.k. Tanaka, f. Toda, *chem*. *Rev*. (2000), 100, 1025.

192. F. Toda, h. Takumi, h. Yamaguchi, (1989) *chem*. *Exp*. 4, 507.

193 f. Toda, k. Tanaka, k. Hami, j. *Chem*. *Soc*., *perkin trans*. (1990) 1, 3207.

194. (a) t. Schmeyers, f. Toda, j. Boy, g. Kaupp, (1998), *j*.*chem*., *sco*. *Perkin trans*. 2, 989. H. Hagiwara, s. Obtsubo, m. Kato, (1996), *mol*. *Cryst*. *Liq*. *Cryst*. 279, 291.

195. M. Tranaka, k. Kobayashi,.(1998), j. *Chem*. *Soc*., *chem*. *Coummun*1965. J. Im, j. Kim, s. Kim, b. Hahu, f. Toda, (1997), *tetrahedron lett*. 38,451.

196.. R.w. Brimblecombe, w.a.m. Duncan, g.j. Durant, j.c. Emmett,c.r. Ganellin, m.e. Parons, (1975), *j. Int. Med. Res*. 3, 86.

197. Y. Tanigawara, n. Aoyama, t. Kita, k. Shirakawa, f. Komada, m.k. Kasuga, (1999), *clin*. *Pharmacol. Ther*. 66, 528.

198. Hunkeler, w.; mohler, h.; pieri, l.; polc, p.; bonetti, e.p.; cumin, r.; schffner, R.w.; (1981), *nature* 290, 514.

199. P. Wasserscheid, w. Keim, (2000), *angew. Chem. Int. Ed. Eng*. 39, 37872; d. Bourissou, o. Guerret, f.t. Ggabbai, g. Bertrand, (2000), *chem. Rev.*100.

200. b. Radziszewski, (1882), *chem. Ber.* 15, 1493.f.r. Japp, h.h. Robinson, (1882), *chem.ber.* 15, 1268.

201. Dye, c., scheele, s., dolin, p., pathania, v., and raviglione, m. C. (1999) *J. Am. Med. Assoc.* **282,** 677–686

202. Parrish, n. M., dick, j. D., and bishai, w. R. (1998) *trends microbiol.* **6,** 107–112

203 duncan, k., and sacchettini, j. C. (2000) in *molecular genetics of mycobacteria* (hatfull, g. F., and jacobs, j. W. R., eds) pp. 297–307, american society for Microbiology, washington, d. C.

204. debus, h., *ann.*, **1858**, *107*, 204.

205 .radziszewski, b., *ber.*, **1882**, *15*, 1493. radziszewski, b., *ber.*, **1882**, *15*, 2706.
Radziszewski, b., *ber.*, **1883**, *16*, 487. radziszewski, b., *ber.*, **1883**, *16*, 747.

206 (a) weidenhagen, e., *ber.*, **1935**, *68*, 1953. (b) weidenhagen, e., *ber.*, **1937**, *70*, 570.

207.wiley, r. H., ed. Pyrazoles, pyrazolines, pyrazolidines, indazoles and condensed rings; in The chemistry of heterocyclic compounds; weissberger, a.., ed.; interscience publishers: New york, 1967; vol. 22, p 180.

208 rajendra prasad y, rajasekhar k.k., shankarananth v, sireesha g,swethaharika k and Poroikov v., synthesis and in silico biological activity evaluation of some 1, 3, 5- trisubstituted-2-pyrazolines. J. Pharm.res, 4(2), 2011, 558-560

209.evaluation of anti-tubercular activity of nicotinic and isoniazid analogues. Arkivoc 2007 (xv), 181-191.

210.maria c. S. Lourenco, marcus v. N desouza, alessandra c pinheiro, marcelle de l.*ferreira, rasnisb b, goncalves, thais cristina m nogneira, monica a peralta.*.